CHAPTER 7

The Nature of the Genetic Effects Produced by Radiation

H. J. MULLER[1]

Department of Zoology, Indiana University

Fundamental properties of the genetic material. Transmission of the genetic material. Effects of radiation on the transmission of the genetic material: Interference with cell division and induction of polyploidy—Disarrangement of cell division and induction of aneuploidy involving whole chromosomes—Effects on crossing over. Consequences of the production of a single chromosome break. Consequences of two breaks in separate chromosomes. Consequences of two breaks in the same chromosome. Structural changes of greater complexity. Nonrandom incidence of the changes produced by chromosome breakage. Position effects induced by structural changes. Influence of stage of cell at time of exposure on the consequences of chromosome breakage. Manner of incidence of radiation-induced and spontaneous mutations of genes. Relative frequencies of different types of character changes caused by radiation-induced and spontaneous mutations. Effects of changing the relative quantities (dosage) of genes. Dominance. Radiation and spontaneous gene-mutation frequencies in Drosophila. Differences between the production of mutations in different species. Agents other than radiation which separately affect mutation frequency. Influence of normal metabolic processes on the occurrence of gene mutations. Relation between mutagenicity and carcinogenicity. Manner of accumulation, expression, and elimination of mutations. Manner of incidence of radiation damage to subsequent generations. Speeding up of evolution by irradiation. Practical applications of the action of radiation on the genetic material. Irradiation of the genetic material as a means of biological investigation: Field of chromosome behavior and properties—Field of gene properties and gene evolution—Fields of development, physiology, pathology, and biochemistry. References.

The gravity of the genetic effects of radiation is of a different order of magnitude from that of all the other biological effects of this agent in that, in the first place, the genetic effects are essentially irreparable. They are therefore also, if repeated, cumulative over an unlimited period. In fact, like all other genetic changes, they tend to be not merely persistent but self-multiplying. They are, in different cases, of utterly diverse kinds, and they range from the mildest to the most radical. The reasons for

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these facts are to be found in the properties of the genetic material itself. These therefore call for a brief preliminary review.

1. FUNDAMENTAL PROPERTIES OF THE GENETIC MATERIAL

Genetic studies on widely diverse organisms have shown that the biochemical operations of the cell, ranging from the most fundamental to the most trivial, and from the "physiological" to the "morphogenetic," depend in the last analysis upon the nature of the self-reproducing nucleoproteins that have been provided. These are, for the most part at least, contained in the chromosomes, although in plants of varied kinds and in some microorganisms of an animal nature some genes have been proved to be present in certain cytoplasmic particles. A *chromosome* has been proved to consist essentially of a fine desoxyribonucleoprotein thread, thousands of times longer than thick, differentiated along its length into hundreds or thousands of functionally distinct and individually self-reproducing regions (probably constituting discrete segments), the *genes*.

Each chromosome, and in fact each gene in it, considered separately, is properly termed self-reproducing, inasmuch as it possesses the property, when in its natural protoplasmic medium, of so guiding the selection and assemblage, possibly the reconstruction and shaping, and certainly the bonding together, of surrounding raw materials, as to result in the construction, next to itself, of an exact copy of itself. Moreover, and most important of all, even if it has undergone some permanent change (*mutation*) in its own inner configuration, it will now guide the next synthesis so that this very change itself also becomes incorporated in the new copy. This property has been called "convariant reproduction."

As a result of this ability to reproduce its own changes, each gene must in the course of ages have undergone an extensive evolution, involving a long series of mutational steps that gave it an increasingly complex organization, more nicely adapted biochemically to serving given needs of the organism. Since mutations are not designed in advance for useful ends, the great majority are necessarily detrimental to survival, and the organisms inheriting these tend to die out. However, the organisms with the relatively rare mutations that happen to be helpful tend to multiply and, because of the ability of the gene to pass on the mutant pattern to its daughter genes, these organisms produce descendants inheriting the advantageous mutation. In addition to this slow step-by-step evolution of each gene there has been, according to modern genetic theory, an even slower, stepwise increase in the number of kinds of genes. The initial event of such a step usually consists in a part of a chromosome, containing a group of genes, becoming detached from its place and inserted into a new position into some chromosome or set of chromosomes which already has these same genes in their old positions as well. This process of form-

ing gene repetitions, called "duplication," is described on p. 377. Once duplicated, the originally identical genes in the two different positions must from then on undergo separate mutations and thereby gradually become differentiated from one another. By these mechanisms it has been possible for evolution to proceed from the stage of one or a few genes, alike and relatively undifferentiated, and producing little or no accessory material, to that of the great constellations of elaborately differentiated, cooperatively acting genes, surrounded by their exceedingly complicated systems of nucleoplasmic, cytoplasmic, and intercellular products, which are characteristic of present-day higher organisms.

There is as yet no good evidence concerning the nature of the mechanism whereby the genes reproduce themselves, or concerning that whereby they control the other biochemical operations of the cell. As regards the former problem, we do not know what level or levels of synthesis are involved in the building of the gene, i.e., whether the stringing together of amino acids, the formation of higher associations, or the foldings of chains are here concerned. We do not know for sure whether it is the nucleic acid polymers, or the protein constituents, or both, which carry the distinctive gene pattern that determines what pattern shall be reproduced. However, the evidence for a virtually pure nucleic acid composition of those gene complexes which undergo transference between cells in *Pneumococci* and certain other bacteria, and which have misleadingly been called "transforming substances," would prove (if confirmed by chemical tests rigorous enough to be generally accepted) that nucleic acid polymers by themselves are capable of carrying the characteristic gene patterns and of acting as determiners for these in self-reproduction.

As regards the problem of gene action on the cell, one line of speculation, starting with Driesch in 1894, has been that they act as heterocatalytic enzymes of the most diverse kinds, thus guiding the course of innumerable reactions. Another line of speculation, originating with de Vries's hypothesis of "intracellular pangenesis" in 1889, has been that, utilizing the same principle as that whereby they reproduce themselves for mitosis, they also produce copies or partial copies of themselves, of protein nature, which, becoming detached, pass out into the protoplasm and there act as heterocatalytic enzymes and/or engage in other reactions, according to the nature of the given gene and of the other attendant materials and circumstances. According to the opinion of some authors, all protein syntheses, and possibly some other syntheses as well, take place only at the site of the gene in the chromosome, by the direct intervention of the gene as a hetero- or autocatalyst; according to others, by the action of gene replicas elsewhere in the cell; while still others would allow these syntheses to be less immediately dependent on the genes. Certainly the composition of each enzyme and of every other protein and, for that matter, of every other substance produced in the cell, depends upon

the activity of given genes, as is shown by the fact that this composition becomes permanently changed by mutations of the genes. Yet it has not to date been possible to demonstrate rigorously that any given enzyme, antigen, or other cell constituent is the direct product, or the partial replica, of some one gene, or that there is, in general, a one gene–one enzyme, or one gene–one antigen relation. In some cases, in fact, the contrary is certainly true.

2. TRANSMISSION OF THE GENETIC MATERIAL

As a result of the reproduction of the genes in the chromosomes, identical twin genes and strings of genes, indistinguishable from each other, forming the basis of *sister chromatids*, come to be present, lying side by side, in the place of each original chromosome. It would probably be more accurate to speak of the two chromatids as "mother" and "daughter" rather than as sisters, although it has not yet been definitely proved that all the original material remains in one (the "mother") gene or chromosome while all the newly gathered material comes to be in the other (the "daughter"). In preparation for cell division by *mitosis* both these chromatids become tightly coiled by helical spiralization, probably of two or more degrees of fineness, into a compact double mass. This structure is appropriate in shape and consistency for having its two members pulled cleanly apart, to opposite poles of the spindle-shaped division figure, with the aid of the tractive "spindle fibers," or lines of streaming, which usually become attached during mitosis to each chromatid at a single fixed point, its *centromere*. In most cases it is only during this contracted mitotic stage that a chromosome may be readily seen and identified. After mitosis, it again undergoes considerable uncoiling and so becomes largely lost to view.

The process of exact doubling and equal distribution, occurring at every somatic mitosis and resulting in the remotest cells of the body receiving chromosomes and genes just like those of the original fertilized egg that existed at the start of development, is interrupted at *meiosis*, in preparation for a new fertilization. Until this stage, in ordinary biparental diploid organisms, all cells have carried along by mitosis two sets of chromosomes, a condition called *diploid;* one of these sets, the "maternal," was contributed to the original fertilized egg by the mother, and the other, the "paternal," by the father. For each maternal chromosome, then, there exists in these cells a homologous paternal chromosome. And, similarly, each gene within a given chromosome is ordinarily matched (except in the case of differing sex chromosomes) by a homologous gene occupying a corresponding position in the homologous chromosome derived from the other parent. But by means of the processes of meiosis, each functional male or female gamete to be formed comes to have only

one set of chromosomes, a condition referred to as *haploid*, instead of two sets, and only one instead of the two genes of each type previously present. For a condensed account of meiosis and its associated genetic phenomena, with diagrams, see Muller, Little, and Snyder (1947), pp. 40–56.

The clue to the complexities of meiosis lies in the remarkable process of *synapsis*, which initiates it. In synapsis each gene in the nucleus somehow finds and becomes temporarily adherent to its homologous gene, usually of identical composition, which was originally derived from the other parent and is still present in the same cell. Since the corresponding genes are usually arranged in the same sequence in homologous chromosomes, this process brings every two homologous chromosomes together in pairs, adhering side by side along the whole of their lengths, with like genes apposed. Each of the members of these pairs now reproduces itself, i.e., it synthesizes a daughter chromatid, so that tetrads, of four chromatids each, are formed in the place of the original pairs. The two meiotic cell divisions follow without any further chromatid formation intervening. Thereby the four members of each tetrad become pulled apart, at their centromeres, to give two and two at the first meiotic division and one and one at the second. Thus they are distributed singly among the four granddaughter nuclei derived from each nucleus that underwent meiosis.

In this way each gamete comes to have just one chromatid—now to be termed a "chromosome"—out of each tetrad, and thus has just one set of chromosomes instead of the original two sets. Accordingly, for every gene of which the premeiotic cell had two representatives, a maternal and a paternal (to be referred to as "a pair" of genes), the gamete carries but one, a maternal *or* a paternal, as the case may be. This is the basis for Mendel's first law, that of *segregation*, whereby 50 per cent of the gametes of any individual receive any given gene inherited from one of the parents, while the rest receive the homologous gene (called an *allele* if it is not identical in composition with the first one) inherited from the other parent.

But a gamete receiving a given gene of one pair does not necessarily receive, as its quota from any other pair, that gene which was derived from the same parent. If the second pair of genes in question lies in another pair of chromosomes, the chance is just 50 per cent that the gene of the second pair which is received by any given gamete is from the other parent than that which furnished that gamete with the gene of the first pair. This is expressed by saying that there is among the gametes a 50 per cent frequency of *recombination* of genes that lie in different pairs of chromosomes. This result is due to the fact that it is a matter of indifference, in the orientation of the tetrads on the meiotic division figures, whether an element of paternal or maternal origin happens to be placed so as to be pulled to a given pole of the division figure, and that

the orientation of one tetrad in this regard does not influence that of another.

If now the second pair of genes being considered should lie in the same pair of chromosomes as the first pair, the frequency of recombination found among the gametes is lower than 50 per cent, and these genes, lying in the two different positions or *loci* in the same pair of chromosomes, are said to be *linked*. That there is some recombination even of genes in the same pair of chromosomes is due to the process of crossing over which occurs during synapsis. This involves the breakage of one of the two paternal and one of the two maternal chromatids of a tetrad at exactly corresponding points, followed by cross-union between the broken ends, in such wise that one of the chromatids formed is a combination derived from the maternal member on one side, say the left, of the point of breakage and reunion, and from the paternal member on the other side, the right, while the other chromatid, complementarily, comes to have a paternal left-hand portion and a maternal right-hand one. Thus if the maternal chromosome had contained the genes A and B and the paternal one the homologous but somewhat different alleles a and b, the two *crossover* chromatids, formed as a result of crossing over between the genes at the two loci, would have the composition Ab and aB, respectively, while the other two chromatids of the tetrad, being *noncrossovers*, would still be of compositions AB and ab, respectively.

In most species crossing over can take place at virtually any point along a pair of conjugating chromatids. Hence, in the case of genes that lie farther apart along the chromosome there will be a tendency to have a higher frequency of crossover combinations formed than in the case of genes that lie close together. Thus the frequency of crossing over can be used, conversely, as an indication of the distance apart of genes, and has lent itself to the plotting of "maps" showing the position of the genes in the chromosome. In these linkage maps, that distance which for convenience is designated as "one unit" is a distance having 1 per cent of crossing over within it. Crossing over can occur in more than one position at a time in a given tetrad, but at distances closer than a given length there is a tendency, called *interference*, which increases with proximity, for crossing over at one point not to occur so readily as usual when there happens to be crossing over at another point. The two chromatids which participate in crossing over at one point can have one, both, or neither of their members the same as those which participate at another point in the tetrad. Both the frequency of crossing over and the amount of interference are influenced by various physiological and genetic conditions, as well as by external agents. They also vary somewhat from one chromosome region to another. In the neighborhood of the centromere, crossing over is much less frequent than elsewhere for a given physical length of the chromosome thread, and it is there

also especially subject to having its frequency influenced by varied conditions.

The primary function of crossing over, as of recombination of genes in different chromosomes, lies in its allowing advantageous mutant genes that arose in different lines of ascent an opportunity to become associated with one another in lines of descent that inherit both or all of these genes at once, without the much longer delay that would usually be required for both or all of them to arise by successive mutations within the same line. In other words, evolution is accelerated by crossing over. In addition, crossing over, tying the four chromatids of a tetrad together by means of the cross figure (*chiasma*) formed at each point of breakage and cross-union serves in most organisms to keep the chromatids from falling apart into the separate pairs that would usually result from their tendency to hold together mainly in twos; thus it facilitates their being so oriented at the meiotic divisions as to segregate in a regular fashion, one chromatid of each tetrad to each gamete.

3. EFFECTS OF RADIATION ON THE TRANSMISSION OF THE GENETIC MATERIAL

3-1. *Interference with Cell Division and Induction of Polyploidy.* The most immediately observable conspicuous effect of radiation on the transmission of the genetic material is its inhibition of mitosis. This effect is produced by all ionizing radiations as well as by ultraviolet, even in very small doses, and it is a striking fact that powerful chemical mutagens such as those of the mustard gas group also produce it. If a cell is already as far along in mitosis as a late prophase, metaphase, anaphase, or telophase stage when the radiation is applied, it will complete its division without interruption; but, if approaching prophase, it will be inhibited from entering this stage for a period of time that may be considerable, depending upon the material and the dose. If in an early or middle prophase it may even appear to regress in phase and will then remain mitotically static until finally cells that had been in interphase during treatment have caught up with it. Thus there is a kind of damming up of mitoses, followed by a burst of them. This crest in the mitotic frequency is in turn succeeded by a trough, since many of the cells that otherwise would have been dividing have then, because of the previous delay, only recently entered interphase. The resulting tendency to synchronization of mitoses is thereafter expressed in a series of gradually subsiding waves of mitotic frequency.

In the fertilization period of the eggs of certain echinoderms, E. E. Just (1926) found that ultraviolet radiation interfered with separation of the polar bodies. The four haploid nuclei then united with one another and with the sperm nucleus to form a *pentaploid* nucleus, i.e., one with five

sets of chromosomes. The later cell divisions were normal except that all five sets of chromosomes were carried along instead of only two, and the cells of the resultant embryo were thus all pentaploid. It is not the rule in material in general, however, either in meiotic or in mitotic divisions, for radiation to cause the union of daughter nuclei which would normally separate. Thus the production of *polyploid* cells or offspring, i.e., those having extra sets of chromosomes, is not a genetic effect of radiation that has been found to be produced generally.

3-2. *Disarrangement of Cell Division and Induction of Aneuploidy Involving Whole Chromosomes.* An effect that has been much more commonly observed, following ionizing radiation at any rate, is the abnormal distribution of chromatids to the daughter nuclei, both at mitotic and meiotic division. When the two chromatids of a chromosome that has doubled for mitosis are carried to the same pole, the process is called "nondisjunction." The same term applies when both pairs of chromatids of a tetrad are carried to the same pole at the first meiotic division, or both chromatids of a pair at the second meiotic division. In any of these cases an equal number of cells is formed with extra and with missing chromosomes, respectively. A related process is that in which a given chromatid or pair of chromatids lags on the spindle in cell division and fails to be carried to either pole. This event is in most material followed by the eventual degeneration of the excluded chromatin. In such cases, cells with missing chromosomes, but not cells with extra ones, are formed.

Both nondisjunction and lagging occasionally occur without irradiation. That they are far more frequent in irradiated material was first shown by Mohr (1919) in a locust, *Decticus*, by cytological methods, and later by Mavor (1921) in *Drosophila* by application of the genetic methods by which Bridges (1913, 1916) had previously demonstrated the spontaneous occurrence of nondisjunction in that material. The induced displacements of chromosomes at meiotic divisions were found to continue for nearly a week after the irradiation had been applied.

Both the addition and the subtraction of a chromosome, but to a greater degree the latter, involve drastic departures from the normal gene ratios and therefore imbalances in the concentrations, relative to one another, of the different gene products. The imbalanced genetic composition, which is designated as *aneuploid* (a term applied generally when any part of the chromatin, whether more or less than one chromosome in extent, is present in the wrong amount relative to the rest of it), is therefore damaging to cell functioning. By proliferation of a cell damaged by aneuploidy, a sector of tissue that is permanently abnormal will be produced. If the affected cell is an embryonic one, it can give rise to an abnormal portion of the body, the size of which will depend upon how large a body region the affected cell is ancestral to. Sometimes the abnor-

mality of the affected cell will be so great as to result in its death or failure to proliferate. The life of an individual as a whole depends on a more complicated and vulnerable organization than that of any part of it, so long as that part has the aid of other, normal parts. It follows from this that, in case a cell with a chromosome extra or missing happens to be in the germ track, so as to give rise to an entire individual rather than just a part of one, such an individual will be especially subject to abnormalities of development and functioning of varied kinds, so much so that it will often be unable to live to maturity; i.e., a *dominant lethal* effect is thereby produced.

In case the extra or missing chromosome is a sex-determining chromosome, however, an individual may be formed which does not show abnormal effects of its gene imbalance, except for the usually minor disturbances caused by an extra or missing Y or W chromosome. Nevertheless, the individual may be of the opposite sex from that which it normally would have been, and so may constitute an exception to the rules by which sex-linked characters are ordinarily inherited. If now the aneuploidy had arisen in only one of the nuclei of an embryo of the two- or four-cell stage, or some other very early stage, only a part (a half, a quarter, or some other fraction) of the individual will have its genetic sex altered. In that case, in organisms which, like insects, have autonomic sex determination of their various parts, a gynandromorph will result. In *Drosophila* irradiation of an egg before its fertilization can so affect its cytoplasm as to cause lagging of chromosomes later, during an early "cleavage" stage, and this is equally likely to affect the chromosomes of either parent whether they had themselves been irradiated or not, as shown by Patterson (1931b). Thus the male portion of the resulting gynandromorph can, as the case may be, exhibit the characters of either the maternal or the paternal sex-determining chromosome.

3-3. *Effects on Crossing Over.* Another way in which the transmission of genes has been found to be altered by ionizing radiation is through its effect on the frequency of crossing over. It was found by Mavor (1923) that in *Drosophila* the frequency of crossing over was decreased by X-ray irradiation in the region of the X chromosome that he was studying, and by Mavor and Svenson (1924) that it was increased in the region that was under their observation in the second chromosome. These influences persisted for more than a week, despite the occurrence of mitosis in the affected germ cells between the time of treatment and the time of crossing over. Some of this crossing over was probably produced in oögonial cells, judging by Friesen's results on spermatogonia (see next paragraph); this would explain a part at least of the apparent persistence of the effect. Studies by the present writer (1925, 1926) showed that the apparent contradiction in the effects on different chromosomes was

in conformity with the principle that, in the regions near the centromeres of all the major chromosomes, crossing over is considerably promoted by irradiation, as others have also shown it to be by extremes of heat and cold, chemical mutagens, and some other environmental as well as physiological and genetic influences, while in regions farther from the centromeres, except perhaps near the very ends, a less marked *decrease* in crossing-over frequency is produced. The decrease is in part, at least, an indirect consequence, resulting from interference, of the promotion of crossing over elsewhere. It may be that the decrease in the inhibiting influence of the centromere on crossing over, caused by radiation, is related to that decrease in the accuracy of transportation of chromosomes which is expressed in their lagging and nondisjunction.

The promotion of crossing over by ionizing radiation (as well as by some other influences) is so strong that it even leads, in *Drosophila*, to occasional crossing over in spermatogonial cells, as shown by Friesen (1933, 1936) and in somatic cells, and in these cases, too, the induced crossing over is mainly, although not exclusively, near the centromere, just where it is of least frequent occurrence normally during meiosis. There is evidence that, as in normal crossing over, this induced crossing over occurring in gonial and somatic cells usually involves only two of four chromatids of a tetrad, and that the two participating chromatids undergo breakage at exactly corresponding points. Since it is not followed by meiotic divisions, the daughter and descendant cells are still diploid. However, they may by this process of crossing over become alike with respect to genes of which the homologous chromosomes originally carried different alleles. Thus if genes in the original paternal and maternal chromosomes are represented as *ABCD* and *abcd*, respectively, the centromere being at or near *D*, a somatic cell may at the mitosis following crossing over receive a noncrossover chromatid with *abcd* and a crossover chromatid of composition *abCD*. In that case the genes *a* and *b*, which previously had been unable to express themselves effectively because of the simultaneous presence of the dominant alleles *A* and *B*, will now be able to produce their characteristic effects, when in the appropriate region of the body and stage of development.

If, then, the crossing over has occurred in an embryonic somatic cell, or in any cell subject to further proliferation, a portion of the body or patch of tissue may thereby come to exhibit recessive characteristics not shown by the body as a whole. This turns out to be, in *Drosophila*, the chief mechanism for the appearance of such patches following irradiation, although it had earlier been thought that they were usually produced by loss of chromosome parts. This is one method by which irradiation can bring preexisting but hidden mutations to light, in a patch of somatic tissue or portion of the body. It is evident that this mechanism might sometimes (when a recessive gene of the appropriate kind is

already present in just one chromosome) lead to the induction of a tumor by radiation. However, it is probable that in most organisms other than Diptera (flies) crossing over would be much less readily induced, if at all, in somatic and gonial cells, than has been found to be the case in *Drosophila*. For Diptera are peculiar in having, even normally, an exceptionally strong tendency to synaptic association of chromosomes in the ordinary somatic cells and in the nonmeiotic germ cells, and this evidently makes crossing over much more readily possible in this material.

The regions of chromosomes near the centromere, and, to a lesser extent, in some material, those near the tips and some other small interstitial regions, are distinguished by their mode of staining and by certain other properties (*vide infra*). They are designated as *heterochromatic*, in distinction from the rest of the chromosome, which is termed *euchromatic*. The promotion of crossing over by radiation, which occurs most markedly in the vicinity of the centromere, extends not only to the heterochromatic region in that location but considerably beyond it, to euchromatic regions for some distance on either side of it, only gradually fading away. Moreover, it is to be found also in some heterochromatic regions derived from the region originally near the centromere when they have by special means been removed so as to be far from the latter.

The influence of radiation on the centromeric and other heterochromatic regions leads to the occurrence of recombination between the homologous or partially homologous heterochromatic portions of the X and Y chromosome even in the *Drosophila* male, despite the fact that in the *Drosophila* male (unlike the male of most organisms) crossing over does not ordinarily occur at all. Thus the radiation in this case results in chromosomes composed partly of X and partly of Y and, by a second step, to combinations of two X chromosomes attached together. Such combinations, which also occur without radiation, as shown by Philip (1935), but with far lower frequency, had previously been ascribed to mere chromosome breakage, to fusion (Stern, 1926), to translocation (Stern, 1927), or to uncompleted division (L. V. Morgan, 1922). It is probable that the line of demarcation between crossing over and structural change of chromosomes, caused by their breakage and recombination at nonhomologous points (see p. 362), is not a sharp one where heterochromatic regions are concerned, since in such regions the genes at different loci behave more nearly as homologues than they do in euchromatic regions. At any rate, there is probably a good deal of leeway in the positions of breakage of the two participating chromosomes, relative to one another, when the "crossing over" is located in a heterochromatic region. Moreover, even the union of pieces is in such regions less orderly, being frequently reverse in arrangement, so that from *ABCD* and *abcd* the combinations *ABba* and *dcCD* can be formed. Such cases therefore may be regarded as transitional to those next to be discussed.

4. CONSEQUENCES OF THE PRODUCTION OF A SINGLE CHROMOSOME BREAK

Much more varied and more important in their consequences for cells, tissues, individuals, and populations, as well as more heuristic, than the effects of radiation on the distribution of whole chromosomes or on crossing over, are its effects in producing *structural changes* of chromosomes, i.e., permanent changes in the linear arrangement of their genes, and in the distribution of genes among different chromosomes. Clear evidence that ionizing radiation produces an abundance of structural changes of varied types, which become reproduced at mitosis and meiosis so as to be inherited by subsequent generations of cells and individuals, was first obtained in *Drosophila* (Muller, 1927, 1928a, b, d; Muller and Altenburg, 1928). These findings were very soon extended to organisms of the most varied kinds, including monocotyledons, dicotyledons, and mammals, by various investigators, among whom should be named especially Goodspeed, Stadler, McClintock, Levitsky, Sax, and Snell. Ultraviolet radiation as well has been found to produce structural changes, but the relative incidence of different types is not the same as with ionizing radiation, and the frequency of structural changes induced by a given dose of ultraviolet is, according to most investigators, much lower than that from ionizing radiation given in such a dose as to match the ultraviolet in the production of gene mutations (see Chap. 8). These peculiarities of ultraviolet will be further discussed in the second volume of this series. The following pages are concerned mainly with the results of breakage induced by ionizing radiations.

Structural changes of the same types as are produced by radiation also arise "spontaneously," i.e., in untreated material, although with far lower frequency. In fact, "spontaneous" examples of most of the types had already been recognized and had to some extent had their conformations determined, largely in *Drosophila* (especially by Bridges, Sturtevant, Mohr, Muller, Stern, and Altenburg) and in *Datura* and a few other plants (especially by Belling and Blakeslee), before the flood of cases contributed by radiation genetics had become available. However, analyses of the cases produced by ionizing radiation, and of the conditions of their production, added much to the understanding both of the pattern of effects produced, and of their mechanism of origination, and from these analyses of the radiation cases a general theory of the process of structural change of chromosomes, whether resulting from radiation or other causes, gradually took shape. It would be beyond our scope here to give an account of the intricate series of steps whereby this theory has been established, but they are to some extent discussed on pp. 363 to 388 and in Chap. 8. The work has utilized some of the advanced techniques of both experimental breeding (involving linkage maps) and cytological

observation (involving, for example, Painter's salivary chromosome methods in *Drosophila* and McClintock's meiotic chromosome methods in maize), combined with radiation techniques.

The primary genetic event in structural change, regardless of the nature of the causative agent or the type of chromosome structure finally formed, has proved to be breakage of the chromosome thread. This interpretation, which had been proposed as only one possibility by the present writer (Painter and Muller, 1929), was first advocated by Levitsky and Araratian (1931) on the basis of their studies on the plants *Crepis*, *Vicia*, and *Secale* and by Stadler (1932) on the basis of his results with maize. It was later supported by findings of McClintock (1932, 1938a, 1939) on the mechanical breakage, through entanglement, of ring chromosomes in maize, by radiation dosage studies carried out on *Drosophila* by Muller in collaboration with Belgovsky and others, using genetic methods (Belgovsky, 1937; Muller, 1938, 1939b, c, d, 1940a; Muller, Makki, and Sidky, 1939), and by Sax and his collaborators on *Tradescantia*, using cytological methods (Sax, 1938, 1939; Sax and Enzmann, 1939).

The broken end of a chromosome thread, fractured either by ionizing or other radiation, by mechanical means or by chemical mutagens (as in the work of Auerbach and Robson), has the property of adhering to another broken end when it meets it and, forming a permanent union, thereby again constituting as continuous a thread as before, which is capable of reproducing itself as such indefinitely. The most usual broken end for the first one to meet is the other broken end derived from the same break. In this case the combination formed is just like the original unbroken thread, and the process is called *restitution*.

If, instead of restituting at once, a broken end fails to join another one before the chromosome reproduces to form two chromatids, then each daughter chromatid fragment has a broken end like that of the mother fragment, and both these broken ends have the property of adhesion. The contact most likely to occur after that is between the homologous broken ends themselves since they, just after their formation, must be nearer to one another than to any other broken ends. In this way chromosomes, called *isochromosomes*, consisting of two identical parts joined mirror-image fashion, are formed (see Fig. 7-1a–d). When these fragments are not provided with a centromere—in which case they are called *acentric*—their union produces an acentric isochromosome, while union between the fragments which are provided with a centromere—termed *centric* fragments—produces a dicentric isochromosome. It sometimes happens, however, in a case in which a chromosome reproduces before it can undergo restitution, that two of the chromatid fragments do later engage in restitutional union, while the others fail to meet one another. In that case the centric fragment will be passed on down

to a daughter cell, and in this next cell generation, on reproducing once more, its two chromatids will usually join to form a dicentric isochromosome.

When a cell containing chromosome fragments divides, any acentric pieces, or acentric isochromosomes, lacking a spindle fiber attachment, fail to become transported to either daughter nucleus. In consequence the descendant cells are aneuploid, lacking this portion of one of their chromosomes, and for this reason are genetically abnormal. If the

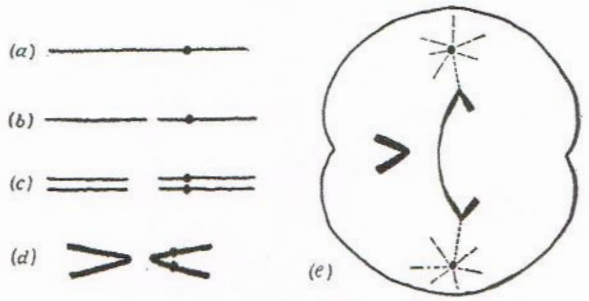

FIG. 7-1. Breakage of a chromosome prior to its splitting, followed by loss of the pieces from the daughter nuclei. (*a*) The chromosome thread before breakage, with the position of the centromere indicated. (*b*) The thread after breakage, composed of an *acentric* and a *centric* fragment. (*c*) The two daughter threads, or "chromatids," have become definitely established before the broken ends can unite. (*d*) Broken ends of twin fragments, being nearer to one another than to other broken ends, join together, forming an *acentric* and a *dicentric isochromosome*, which are now becoming more condensed. (*e*) In the ensuing cell division, the acentric isochromosome fails to be pulled to the poles, and the dicentric one, pulled both ways, tends to form a bridge. If this becomes broken by the tension, it repeats the process of dicentric formation, and another bridge results at the next division, and so on, until the chromosome is lost or the cells are killed.

missing part is large and important enough this deficiency can even cause their death. As mentioned before in connection with the loss of a whole chromosome, such a defect in a germ cell would be especially likely to cause the abnormality and the death of the individual derived from that germ cell.

As for the dicentric isochromosome that has been formed, its two centromeres at the next mitosis are oriented toward opposite poles, just as they would have been if the chromatids had not undergone breakage and union, and the chromosome is thereby pulled in both opposite directions at once (see Fig. 7-1e). Thus the dicentric isochromosome may fail to enter one or both nuclei, and the resultant nucleus or nuclei, lacking also the acentric portion, are rendered deficient for all of the broken chromosome. Thereby the cells are caused to be more abnormal than if they had lacked only the acentric part. Another complication is that the dicentric isochromosome, in being pulled both ways, tends to form a

bridge between the daughter nuclei, and this bridge may later, through a mechanism not yet well understood, lead to the death of the cells involved. This has been shown to happen even when their genetic deficiency is compensated for by supplying them with an additional chromosome of appropriate type (Pontecorvo and Muller, 1941; Muller and Pontecorvo, 1942b; Pontecorvo, 1942).

Sometimes, instead of failing to enter the nuclei, or forming a fatal bridge, the isochromosome stretched in the bridge becomes broken again, and one or both of the two fragments may then be pulled into their respective nuclei. Thereafter, their own daughter chromatids, because of their broken ends, repeat the story of dicentric isochromosome and bridge formation. This process is in the animal material studied unlikely to go through many cycles without the affected chromosome finally becoming lost from the descendant cells, or else killing them by bridge formation. In some material, e.g., maize, the above "breakage-fusion-bridge cycle" may be repeated almost indefinitely, as McClintock (1932, 1938a, 1939 et seq.) has shown. In this case, since the chromosome is broken anew at every mitosis, and since each new break is likely to be in a different position than before, the genetic composition of the repeatedly patched remainder becomes more and more abnormal, as some chromosome parts are lost while others become increasingly reduplicated. Accordingly, the genetic composition of the descendant cells comes to depart ever further from the normal, to their increasing detriment.

Thus, when breakage of a chromosome is followed by union between identical "sister" (mother and daughter) fragments to form isochromosomes, genetic deficiencies and sometimes other genetic abnormalities inevitably follow, by one means or another. If this happens in the germ track of the main or sporophyte generation of a higher plant belonging to an ordinary diploid species (having two complete sets of chromosomes in that generation), and if the affected cells manage to survive until the haploid or gametophyte generation (that with one set of chromosomes), those with the deficiency are then killed off in the latter stage. At least this is true in the male gametophyte, since this metabolizes more completely on its own account than the female gametophyte does and hence has more use for its genes. For now this tissue no longer has a second, normal set of chromosomes to mitigate the effect of its genetic abnormality. In this way the gametophyte generation (at least that of the male, and to a lesser extent that of the female) serves as a sieve to weed out such cases.

In animals the corresponding haploid stage, found in the gametes, does not perform a like selective function, since the limited type of metabolism of these cells does not depend upon their genes, which are in a dormant state at that time, but upon the products of the genes present before reduction, and of the genes in diploid supporting (nurse and

Sertoli) cells (Muller and Settles, 1927). However, as has been stated above, the defect will usually—unless the missing chromatin is exceptionally small or unimportant—cause abnormalities sufficient to kill the individual of the next generation at an early stage of its development. This occurs despite the fact that its cells, being diploid, contain one normal set of chromosomes.

Convincing evidence has been reported that in some material, for example, maize sporophytes (McClintock, 1939), a chromosome broken by ionizing radiation which fails to make contact with another broken end may after a time undergo *healing*, in that the broken end permanently loses its adhesive property so as to be able to function like a normal unbroken chromosome end. If this occurred, the centric fragment could reproduce itself without danger of forming a dicentric isochromosome and so becoming lost or killing the cell. It would therefore be carried along in mitosis like any other chromosome. But the cells with this chromosome would nevertheless be deficient for the acentric fragment, having what is called a *terminal deficiency* of the affected chromosome. They would therefore be genetically abnormal, and this deficiency would still be enough, in the vast majority of cases, to kill the gametophytes. Ultraviolet light has been reported by Stadler (1939) and by Swanson (1942) to be especially conducive, in some plant material, to producing this effect. In animals, on the other hand (and also in maize gametophytes and endosperm when the breakage is mechanical or by ionizing radiation), the evidence is all against the occurrence of healing, despite some contrary claims. It may be concluded that, at least in those animals studied, the free unbroken ends of each chromosome have a characteristic structure, which a broken end cannot ordinarily assume, and that this structure is necessary for the continuance and orderly distribution of the chromosome through repeated mitoses. This property justifies us in distinguishing the normal free ends in such organisms as *telomeres*, in contrast to the interstitial portions of the chromosome, which include the centromeres.

5. CONSEQUENCES OF TWO BREAKS IN SEPARATE CHROMOSOMES

When two chromosome breaks have occurred in the same nucleus, each of the breaks may be followed by one of the types of behavior pattern already described. However, the alternative possibility now arises of one broken end meeting and forming a union with an end derived from a different break. The type of rearrangement which thereupon results depends on where in the chromatin the breaks are located and which broken ends unite with which.

If, as is more often the case, the two breaks in question occur in different, nonhomologous chromosomes, and a fragment of one of these chromo-

somes then unites by its broken end with a fragment of the other, the event—as well as, by an elision of speech, the resulting chromosome configuration—is called a *translocation*. It is probable that in many of these cases the other two fragments fail to find each other. If these uncombined fragments should later undergo healing or if they should finally have their mother and daughter chromatids unite with one another to form acentric and/or dicentric isochromosomes, the descendant cells would be aneuploid, provided they had survived bridge formation. In this case (supposing that these events took place in germ cells) it is very unlikely that the chromosome which did undergo the translocation would be able to get as far as the adult stage of the next generation.

It often happens, however, that the broken ends of the other two fragments likewise find one another and form a union. Such a case is known more specifically as one of *mutual* or *reciprocal translocation* or *segmental interchange* (these three terms being synonymous), and when the word translocation is used without qualification it usually refers to one of this type. In the formation of such a translocation, it is largely a matter of chance whether (1) the centric fragment of one chromosome happens to join the centric fragment of the other, so as to form a dicentric chromosome, while the other two fragments on uniting form an acentric chromosome, or (2) each centric fragment joins on to the acentric fragment of the other chromosome (see Fig. 7-2, 1–3a, b). The first contingency gives rise to what is called an *aneucentric* configuration, and eventually leads to the loss of all parts concerned, and therefore to the loss of all the material of both original chromosomes, by the mechanism already explained for acentric and dicentric chromosomes—unless before this happens it kills the descendant cells by bridge formation (Fig. 7-2, 3a–5a). If any fertilized eggs were thereby produced which lacked two chromosomes, they would, in the great majority of species at least, excepting some polyploids, die at an early stage of embryogeny. The second contingency, on the other hand, involving a *eucentric* configuration, results in two monocentric chromosomes, both of which are transported in a regular manner at mitosis (Fig. 7-2, 3b–5b). In this case all descendant cells derived by mitosis from the cell in which the translocation occurred contain all the chromosome and gene material which is normally present. This second contingency then provides translocations which can be transmitted to descendants, which are viable, i.e., able to survive, and which can be bred and studied genetically.

The descendants, inheriting the two translocated chromosomes from the parents having the aberration, and two normal chromosomes, containing homologous genetic material in its original arrangement, from their unaffected parent, do not themselves suffer from abnormalities caused by the structural change (except in the cases, very rare for most species, of "position effect," discussed in Sect. 9). For they possess two

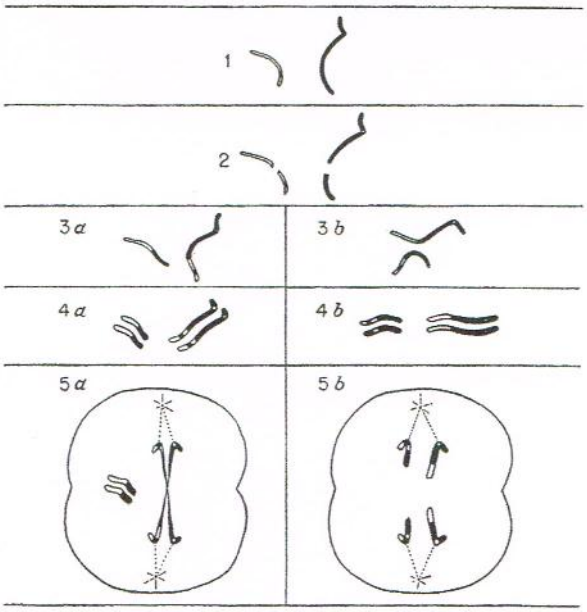

FIG. 7-2. Translocation produced by breaks in two different chromosomes. (1) Two nonhomologous chromosomes are shown, prior to their splitting, one represented in white with a black centromere and the other in black with a white centromere. (2) Two breaks have been induced by radiation, one in each chromosome. (3*a*) The two centric pieces have united to form a dicentric chromosome, and the two acentric pieces to form an acentric chromosome. (3*b*) The alternative type of rearrangement has occurred, giving monocentric translocated chromosomes. (4) The chromosomes have split to form "daughters" or chromatids, and are becoming condensed and placed in position for cell division, in both the *a* and *b* cases. (5) Mitosis is nearly completed. In case 5*a* the dicentric daughter chromosomes are forming bridges and are not being properly pulled all the way to opposite poles (they may later be broken again by the tension), while the acentric daughter chromosomes fail to be transported at all. In case 5*b* the translocated daughter chromosomes become properly transported, so that each of the two daughter nuclei receives a complete outfit of the original chromosome material that underwent translocation, although in a new arrangement. It should be noted that in case 5*a* it will not always happen that the two centromeres of a dicentric chromosome are pulled to opposite poles, since they are not symmetrically placed; but if this does not chance to occur at the first mitosis after the translocation process, it is bound to occur at some subsequent mitosis and so the same kinds of effect as in case 5*a* will finally be produced, and will involve the loss of two chromosomes and the production of two bridges instead of one.

complete sets of chromosomes and of genes, that is, "balanced" gene ratios. Yet, when their germ cells begin to mature and to enter the stages of meiosis, their translocated chromosomes, in matching their homologous parts in synapsis with the nontranslocated ones, have to make a kind of cross figure (see Fig. 7-3a_1, a_2). In this (if we may simplify the situation so as to show only one meiotic division, with one

chromatid per chromosome and no crossing over), it is in most species, for most translocations, more or less a matter of chance whether they assume the "old combination arrangement," as in Fig. 7-3a_1, b_1, such that the two translocated chromosomes become pulled to one daughter nucleus and the two nontranslocated to the other nucleus, or the "recombination arrangement," shown in Fig. 7-3a_2, b_2, whereby one translocated and one nontranslocated chromosome become pulled to each nucleus. It is only when the points of breakage had been very near the centromere

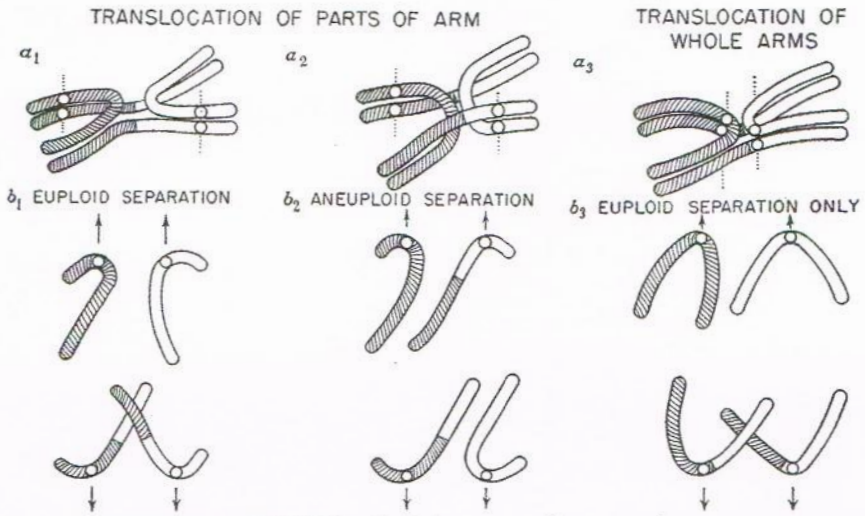

FIG. 7-3. Meiotic arrangements in cells heterozygous for eucentric mutual translocations. In columns 1 and 2 the translocation involved the exchange of parts of arms, while in column 3 virtually whole arms were exchanged. Clear areas represent centromeres. Diagrams simplified by omission of crossing over and of split into chromatids.

in both chromosomes, giving what are called *whole-arm translocations*, that the recombinational arrangement (that giving aneuploids) is of very infrequent occurrence, since here the two nonhomologous pairs of centromeres are so close together that the orientations of their members with respect to the plane of separation are strongly correlated with one another (Fig. 7-3a_3, b_3).

Now the first arrangement, that giving the "old combinations," results both in apparently normal offspring which, however, like the parent, again contain the translocation, and in entirely normal offspring. But the second or recombinational arrangement results in aneuploid zygotes which, being deficient with regard to one translocated chromosome region and at the same time having an extra representation ("duplication") of another region, usually die of their genic imbalance during an

early embryonic stage. Thus the individual with a translocation, though appearing normal, is usually capable of producing only about half as many viable (surviving) offspring as a really normal individual can. In consequence, in most species, if a number of individuals with translocations have arisen in a given generation, this number will be reduced to approximately a half, an eighth, a sixteenth, etc., in successive generations, until at last the translocations (with the possible exception of whole-arm translocations) have completely died out.

In primates, however, where there is but one foetus per pregnancy and its early death in utero is rather promptly followed by another pregnancy, this process tends to compensate for the deaths and thus to allow the so-called "semisterile" individual with the translocation (or, if this individual be a male, his mate) to bear nearly as many offspring as an entirely normal individual does. This will greatly delay the dying out of the translocation. In man, especially civilized man, the additional compensatory factor enters in that a couple subject to involuntary abortions or miscarriages consciously tries to bring their total number of offspring up to or even beyond the average number. In this way the translocation, along with the "semisterility" occasioned by it, must become actively perpetuated.

When two breaks occur in *homologous* chromosomes of a diploid cell, they will usually be at nonidentical points. If all broken ends succeed in uniting, but in such a way that the newly constituted chromosomes are made of one portion from one homologue and the other portion from the other, we again have the possibility of the configuration being either aneucentric (with acentric and dicentric chromosomes) and so becoming lost, or eucentric (with monocentric chromosomes) and so allowing the daughter chromosomes ("chromatids") to be transmitted regularly at succeeding mitoses. In the latter case one of the newly constituted chromosomes will be deficient for some genetic material, while this material will be twice represented (duplicated) in the other new chromosome. The cells derived by mitosis from this cell have the normal number and kinds of genes, however, since the lack in one chromosome is exactly complemented by the duplication in the other. But if this unequal exchange between homologues has occurred in a germ cell, then, after its cycle of mitoses has been completed and it undergoes the meiotic divisions, a gamete is finally produced which has just the deficiency or just the excess of genes. The zygote resulting from such a gamete is therefore aneuploid: it has an "imbalanced" gene content, since its genes are in abnormal ratios to one another, and it is correspondingly abnormal. Only very small deficiencies are compatible with the life of an individual as a whole, even when the homologous chromosome (that which was received from the other parent) is a normal one. Considerably larger duplications than deficiencies can usually be tolerated but, depending on

their size and contained genes, they too tend to cause morphological and physiological abnormalities.

Deficiencies and duplications can be formed similarly as a result of breakage of two "sister" (mother and daughter) chromatids in different positions, when this is followed by eucentric interchange of segments between them. In this case, however, the immediately following mitosis will cause one daughter cell to receive the deficient and the other the duplicated chromatid, instead of both complementary combinations.

It has been explained on p. 365, in connection with the formation and loss of acentric and dicentric isochromosomes following single chromosome breakage, that plant nuclei which are deficient for a portion of chromatin tend to die out in passing through the stage of the male gametophyte generation, since this is haploid, and requires the active functioning of its whole set of genes, and that to a lesser extent there is a similar elimination in the metabolically less active female gametophyte stage. This same principle also operates to eliminate in the gametophyte stage the nuclei, deficient in one or more chromosome regions, which are formed as a result of translocation, or as a result of meiosis in individuals carrying translocations. Duplication of regions is also more likely to be fatal in this stage than in the diploid stage, since it occasions more pronounced genic imbalance when in a combination which is mainly haploid than in a diploid. Similarly, the aneuploid combinations resulting from other types of structural changes, to be described in the next two sections, tend to be eliminated in the gametophytes of plants. In animals, on the contrary, since the genes are not functioning in the gametes that carry them, all aneuploid combinations succeed in being transmitted to the zygotes. They may or may not then cause death or abnormality of the offspring, depending upon the drasticity of the genetic departure of the zygote from the normal diploid combination. So, for example, fully viable zygotes can readily be formed in animals, when a mating occurs between individuals both of which carry the same type of translocation, by the union of one gamete having one aneuploid combination with another gamete having the complementary combination, whereas this could hardly happen in plants.

6. CONSEQUENCES OF TWO BREAKS IN THE SAME CHROMOSOME

When two different breaks occur in the same chromosome, two end fragments and a middle fragment are formed. Which one of these bears the centromere depends on the morphology of the original chromosome and the location of the breaks. If one of the end pieces bears the centromere, and this piece unites by its broken end with the broken end of the other end piece, a process called *deletion*, a *deleted* chromosome, lacking the middle section, is produced. This deficient chromosome will be

transported in normal fashion at mitosis, while the excised middle piece itself, being acentric, will fail to reach either of the daughter nuclei. Thus the descendant cells will be aneuploid, in that they are deficient for this section (see Fig. 7-4a).

The degree and type of damage to the descendant cells caused by the deficiency will, just as in the above-considered cases of deficiencies occasioned by translocation between homologous or sister chromosomes, depend upon the size of the deficiency, the importance of the missing genes, and the extent to which those present in the undamaged homologous chromosome are able in "single dose" to perform the biochemical functions which are ordinarily carried out by the two doses present in normal cells. An offspring derived from such a deficient cell, even though provided with a normal homologous chromosome from its other parent, usually dies of its genic imbalance (abnormal gene ratios) unless the deficiency is a very small one. However, an extremely small deficiency of only one member of a pair of homologous chromosomes may result in no perceptible abnormality. The deficiency will in that case behave in the same way as a "recessive lethal" (see pp. 394 and 403). It may be inherited by a succession of descendants. Having a slightly weakening action on them, it will tend to cause the line of individuals containing it to die out. If, however, it happens to persist long enough, a mating will finally occur, in some subsequent generation, between two apparently normal parents both of which have one deficient chromosome of the given kind. Then an individual can be produced which receives the same deficient chromosome from both parents, and this individual will be killed outright by its genetic abnormality. Thus in any event the deficient chromosome finally dies out.

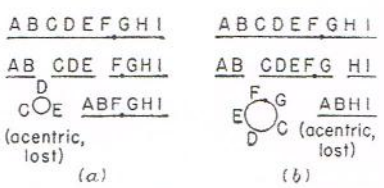

FIG. 7-4. Two breaks in one chromosome, with all broken ends uniting so as to give rise to a deleted and a ring chromosome. In (*a*) (on left) the deleted chromosome, being centric, can continue, while in (*b*) (on right) the ring is centric and can continue provided it is not twisted on its axis.

Exceptions to the rule of the lethality of any deficiency inherited from both parents are provided only by those extremely rare cases in which the deficiency comprises only one or a very few genes, none of which happens to be indispensable for survival. An individual with such a deficiency in both homologous chromosomes might live, but would probably evince its condition by one or more biochemical or morphological inadequacies. These, though not directly lethal, would hamper it, so that in time—though not so quickly as with a lethal—it too would undergo extinction.

If the chromosome broken into three parts had had its centromere in the middle section, then the two end pieces on uniting would have formed

an acentric chromosome that failed to reach the daughter nuclei (see Fig. 7-4b). However, the middle piece might in this case be able to survive for a time, provided its two broken ends happened to become bent around so as to touch and unite with each other, forming a ring or "closed" chromosome. At least, it could survive if in this process the chromonema (chromosome thread) had preserved its axial orientation, but, if one end had become twisted by one or more complete turns, relative to the other end, then when the ring chromosome later reproduced to form two ring chromatids these would find themselves interlocked and hence incapable of being transported to the daughter nuclei (unless they broke again—an event which might lead to further complications). The rings formed without torsion would not be subject to this difficulty, but any descendant cells or individuals that inherited such a ring would, of course, be deficient for both end pieces of the chromosome. Whether they could survive for a time despite their abnormality would then depend on the size and importance of the resulting gene imbalance.

Since the regions of chromosomes in the neighborhood of their ends, in *Drosophila* at least, are composed of heterochromatin (see Sect. 3), which is more or less dispensable, a few cases of rings with only very tiny end deficiencies are known, which result in apparently normal individuals even when both the homologues of the given chromosome possessed by the individuals are of this ring type. Nevertheless, these as well as all other ring chromosomes tend eventually to die out in the course of breeding of a population. This is because a ring chromatid, when it undergoes single crossing over with its partner at meiosis, necessarily gives rise (no matter whether the partner chromatid is itself a ring or of normal structure) to a dicentric chromatid that fails to be transported properly to the daughter nuclei. As a result, fewer germ cells capable of developing into normal offspring are formed by individuals with rings than by those with only non-ring chromosomes, and this reproductive disadvantage leads all lines of descendants with rings eventually to become extinct.

The reproductive disadvantage occasioned by the formation of dicentric crossover chromatids is not so great as might be thought. As Sturtevant and Beadle (1936) have shown, this is because, when crossing over occurs in the meiosis of the oöcyte between two chromatids of a tetrad and not the other two, any dicentric chromatid resulting, being pulled toward both poles at once, tends to become stalled near the middle of the spindle of the first meiotic division, leaving the two noncrossover chromatids to be pulled to opposite poles, one entering the inward-lying nucleus that is destined to form the egg. Then, at the second meiotic division, this inner noncrossover chromatid, which is still in partial conjugation with the dicentric one but more centrally placed than the latter, becomes separated from it in such a way as to be pulled still further in, into the

egg ("oötid") nucleus itself, while the dicentric is left outside. In this way the egg comes to receive a noncrossover chromatid, which has an equally good chance of being either a ring or a non-ring if the individual had had both types to begin with. However, in some cases crossing over occurs at more than one point in a tetrad, and, when this crossing over happens to involve three or four of the chromatids of a tetrad, an egg nucleus can be formed which fails to receive either a ring or a non-ring derived from this tetrad. This result, leading to aneuploidy among the offspring, is of course reproductively disadvantageous, although the incidence of the disadvantage is considerably lower than that for most translocations.

When now we consider meiosis in the male, where all four nuclei derived from each spermatocyte enter actual gametes instead of (as in the female) being arranged in the form of one inner gamete nucleus and three outer, polar body nuclei, it is evident that any crossover dicentric chromatids must result in two deficient gametes, and/or gametes aborted by bridge formation. Thus in the breeding of the male the reproductive disadvantage conferred by a ring chromosome would in most organisms (including mammals) be high. However, in *Drosophila* and any other species (such as many Diptera) in which crossing over did not occur in the male this disadvantage would be avoided.

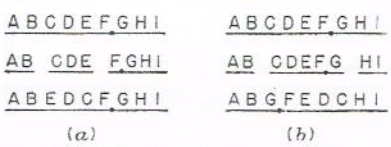

FIG. 7-5. Two breaks in one chromosome, with all broken ends uniting so as to give rise to an inversion. In (*a*) (on left) the inversion is paracentric, in (*b*) (on right) it is pericentric.

Another method of union of broken ends of a chromosome which has undergone two breaks is for all three resulting chromosome pieces to join together again, with the middle piece still in the middle but facing in the opposite direction, with regard to the end pieces, than that in which it was before. This event, as well as the result, is called *inversion* (see Fig. 7-5). It would be equally correct to describe it by saying that the two end pieces interchanged positions with one another. If the centromere is in one of the end pieces, the inversion is called *paracentric;* if in the middle section, *pericentric*. In either case, all parts, including the centromere, are present in the reconstituted chromosome, and its chromatids are properly transported to the daughter cells at each mitosis. Hence it does not (except in the special case of "position effect," discussed in Sect. 9) result in damage to the descendant cells or individuals which inherit it.

However, when crossing over occurs between the chromosome with the inversion and its homologue of normal structure, within the region of the inversion, aneuploid crossover chromosomes are produced, having a deficiency of one region and a duplication of another. In the case of

pericentric inversions these aneuploid crossover chromosomes are monocentric and therefore become transported regularly to the daughter nuclei. The resulting gametes then give rise to genically imbalanced offspring, which are usually unable to survive or are at least grossly abnormal. This puts pericentrically inverted chromosomes, like translocated and ring chromosomes, at a reproductive disadvantage and causes their eventual extinction.

In the case of crossing over within paracentric inversions, the crossover chromatids likewise have a deficiency and a duplication, but in addition one of the crossover chromatids is dicentric while the complementary one is acentric. As we have seen happen with dicentric rings, these crossover chromosomes tend to be left near the middle of the meiotic spindle so that the egg becomes provided, instead, with one of the monocentric noncrossover chromatids (equally often the inverted and the noninverted one). Thus the genetic damage to egg nuclei tends to be circumvented, unless more than two chromatids of the tetrad have all undergone crossing over within the inverted region. In the male, however, where all four nuclei enter into gametes, each occurrence of crossing over within the inverted region must result in at least two gamete nuclei that are either deficient for a whole chromosome or, what is worse, that contain a dicentric chromosome, or that become aborted by bridge formation. Thus the reproductive disadvantage of paracentrically inverted chromosomes, leading to the genetic extinction of these chromosomes and of the lives of descendants containing them, must work especially through the males, as with ring chromosomes. But again an exception must be made of *Drosophila* and those other relatively rare species in which there is no crossing over in the male. In them paracentric inversions can survive rather freely, and they have, in fact, become common in some populations of such species, apparently without detriment to the latter. In fact, in such cases, it is sometimes advantageous for the species to have at its disposal structurally different alternatives of a given type of chromosome, each alternative being provided with a set of genes adapted to a somewhat different type of situation. This gives one basis for divergence in the evolution of adaptations.

The smaller an inversion is, the less frequently will crossing over occur between it and a noninverted chromosome within the region of the inversion; hence the smaller will be its reproductive disadvantage and the slower its extinction. Very small inverted regions probably never have an opportunity to synapse with their noninverted homologous regions, since in these cases the conjugation of the rest of the chromosome on each side tends to prevent the inverted regions from attaining the necessary alignment of corresponding parts with one another. In consequence, these small inversions do not suffer from any appreciable reproductive disadvantage and they sometimes survive indefinitely.

7. STRUCTURAL CHANGES OF GREATER COMPLEXITY

The larger the number of breaks, the higher is the probability that nonrestitutional union of the pieces will result in one or more acentric, dicentric, or polycentric chromosomes and thus result in the death or extreme abnormality of descendant cells or individuals. The euploid changes (those involving neither deficiency nor duplication) that result from just three breaks are the following:

From breaks in three different chromosomes, (1) translocation by triple exchange of a rotational nature, a piece of A becoming attached to the "stump" (centromere-bearing fragment) of B, of B to the stump of C, and of C to the stump of A. From two breaks in one chromosome and one in another, (2) mutual translocation, with an inversion adjoining it in one of the chromosomes, or (3) translocation of the nonmutual, *deletion-insertion* type, a piece being deleted from chromosome A and inserted into chromosome B at the point where B had been broken. From three breaks in the same chromosome, (4) a result denoted as *shift*, involving the interchange in position of the two interstitial fragments, each in the place of the other, with or without the inversion of either one; this is, in effect, deletion-insertion within one chromosome.

Rearrangements have also been found which involve many more than three breaks. Some of them are extraordinarily complex, but in all cases they can be described as combinations of the various types of structural changes already set forth.

In the case of all four of the euploid types of rearrangements resulting from three breaks, offspring receiving them will ordinarily (barring "position effect") appear normal, since there is no genic imbalance. However, in all cases, meiosis in the offspring carrying both the rearranged chromosomes and (from their other parent) the normal homologues will result in the production of some aneuploid gametes, the zygotes derived from which will die; thus these types will be at a reproductive disadvantage, leading to their extinction. In the first three of the above four types the aneuploid combinations will arise by means of the recombination of entire chromosomes, just as happens with ordinary translocations, and the frequency of these aneuploids will usually be very high. In the last type, the shift, the aneuploid combinations are formed by crossing over, and their frequency will therefore be lower, depending mainly on the length of the larger of the two pieces that were interchanged in position. The rearrangements involving more than three breaks, like those involving three, are almost always, when capable of surviving at all, subject to reproductive disadvantages in later generations, and hence these also tend to die out.

In the discussion of structural changes involving more than one break, only those types have been examined above in which the union of pieces

preceded the reproduction of the chromosome to form chromatids. However, in the section on the consequences of a single break it was pointed out that union of the broken ends sometimes fails to occur until after the fragments have reproduced. It was seen that in some of these cases one of the chromatids may undergo restitution while the other, failing to do so, results in acentric and eventually also in dicentric chromosomes. Similarly, when two or more breaks have occurred the union of some or all of the broken ends may be delayed until after the pieces have reproduced to form chromatids. Here, too, the sister fragments may thereafter follow different courses so that, for example, a chromosome broken at two places may give rise to one chromatid with an inversion and one with a deletion having its breaks at the same points as those of the inversion. Moreover, the chromatid fragments may become transferred in such a way as to result in one daughter nucleus having a deficiency while the other receives a duplication, which may be attached either to the homologous chromosome that already contains (often in an adjoining position) another representative of the same chromosome region, or to a nonhomologous chromosome. The production, in this manner, of daughter cells of different but often more or less complementary genetic types, derived from one treated cell, is seen especially strikingly when spermatozoa have been irradiated, for then a visibly mosaic individual may be formed, approximately half of which is descended from each of the two genetically different daughter cells of the "first cleavage" stage.

8. NONRANDOM INCIDENCE OF THE CHANGES PRODUCED BY CHROMOSOME BREAKAGE

The frequencies with which structural changes of different types are found following irradiation do not follow a purely chance distribution, even when due allowance is made for the fact that some types are much more subject than others to elimination before being found. The most marked irregularity in distribution is to be noted in the great excess of structural changes involving one or more breaks in a heterochromatic (see last part of Sect. 3) region of a chromosome, when we take into consideration how very short the heterochromatic portions of the chromosome threads are in comparison with the euchromatic parts. Most of the heterochromatin of a normal chromosome lies in a short region on either side of its centromere, and a very little adjacent to each of its free ends or telomeres (Prokofyeva-Belgovskaya, 1937, 1938; Muller, 1938); yet a high proportion of all the radiation-induced translocations, inversions, and deletions formed in *Drosophila* have involved one or more breaks in a heterochromatic region, usually in a region near the centromere. So, for instance, in the X chromosome of *Drosophila* the heterochromatin near the centromere occupies only about a twentieth the

length of the chromosome, as seen in the extended interphase stage represented by the chromosomes of the salivary gland, whereas something like a third of the translocations undergone by this chromosome have had their break in its centromeric heterochromatin.

It is true that in the condensed X chromosome, as seen during mitosis, the heterochromatin in the neighborhood of centromeres does occupy about a third of its length (Muller and Painter, 1932), and that a similar relation holds in the case of the other chromosomes, but this is due to the large size of certain chromatin accretions called *blocks* (Muller and Gershenson, 1935) which are embedded in the heterochromatin in the neighborhood of the centromere at this stage. These blocks are of the nature of adventitious nongenetic material (though produced under the influence of certain genes in their vicinity), and they do not indicate a corresponding length of coiled "gene-string" within. Moreover, breaks are seldom if ever produced within these blocks themselves. Now it has been found that when the genes for the blocks are removed, by means of a structural change, to some other chromosome region, the heterochromatin which has been separated from them is still as susceptible to having structural changes induced in it as before, although it now occupies about as small a fraction of the mitotic chromosome as it does of the salivary gland (interphase) chromosome (Muller, 1944). Similar observations have shown that this susceptibility of the heterochromatin does not depend upon the presence of either the centromere or the nucleolus in its vicinity. It is therefore a property of the heterochromatic region itself.

This susceptibility of heterochromatin to structural change does not necessarily mean that it is more easily broken by radiation. It is quite possible that it is broken no more readily than euchromatin is, per unit of length of its chromosome thread, but that it is much less likely than euchromatin to undergo restitutional union of its broken ends as compared with union between parts that were not together before. This might be either because its broken ends became adhesive later than those of euchromatin, during the period when the condensed chromosomes undergo extension for interphase, or, more likely, because they were more subject to movement. The centromeric heterochromatin is often under special tension, which would tend to move its pieces apart if broken. Moreover (as mentioned in the last part of Sect. 3) any heterochromatic region tends to undergo conjugation with any other heterochromatic region, even if the other does not lie in a homologous chromosome position, and so these regions, in *Drosophila* at any rate, are probably more subject than euchromatic regions are to forces of attraction which tend to move them about. Further evidence pointing in this direction appears to be provided by the finding of Prokofyeva-Belgovskaya (1939; Prokofyeva-Belgovskaya and Khvostova, 1939), confirmed by Kaufmann (1939), that there are even within the euchromatic portions of *Drosophila*

chromosomes a considerable number of regions which are much more susceptible than the rest to becoming involved in structural changes, and that, according to the former investigator, these very regions show somewhat of the same tendency to conjugation with other such regions, as well as with centromeric heterochromatin, as is shown by the heterochromatin proper. She regards these interstitial regions, therefore, as having a composition somewhat like that of heterochromatin. Still further evidence of the effect of movement in promoting structural change is to be found in Sax's (1942) finding that already acentric pieces, produced by a prior irradiation, are much less likely than centric ones to undergo further structural change when radiation is again applied.

Not only are certain chromosome regions more likely than others to engage in structural change, but it is found, when the frequency of different structural changes is tabulated, that they show some tendency for structural changes involving a break in one position to have their other break in the same chromosome arm rather than elsewhere. This is true despite the finding of Sax, just mentioned, that a break distal to another one is in some circumstances more likely to restitute. Thus, in chromosomes of *Drosophila* with two arms (regions on each side of the centromere) of approximately equal length, the ratio of inversions in which both breaks are in the same arm (paracentrically) to those in which they are in different arms of the same chromosome (pericentrically) or in other chromosomes (translocations) is about twice as high as would be expected on a chance distribution, as shown by Catcheside (1938), by Bauer, Demerec, and Kaufmann (1938), and by Bauer (1939). No doubt this is in part an expression of the fact, to be discussed in Sect. 10, that there is a distance limitation on the position, at the time of breakage, of broken ends which will be able later to undergo union with each other; this at the same time indicates that the movements of ends relative to one another, between the time of breakage and that of union, are rather limited. In spite of this there is no pronounced tendency for the distribution of inversions of different lengths, yet long enough to be readily discovered by cytological means, to depart from that expected on a random basis (Bauer, Demerec, and Kaufmann, 1938; and Bauer, 1939).

There does, however, appear to be a distinct tendency for structural changes in which the breaks were very near together, resulting in minute deletions, insertions, and inversions, to be more numerous than expected on a chance basis. In part their apparently higher frequency is due to the selective factor that, in the case of deletions (the largest class among those readily found), the more minute ones survive better, but in part the discrepancy in numbers seems to be a real one. It is probably to be explained not only by the spatial limitations on union of broken ends already mentioned, but also by a tendency for breaks to occur close together, occasioned by the manner of operation of the radiation in

causing breakage, discussed in Chap. 8. At any rate, the result is so marked that it becomes justifiable to speak of "minute rearrangements" as a class more or less to be distinguished from, although overlapping with, that of "gross rearrangements" (Muller, Prokofyeva, and Raffel, 1935a, b; Muller, 1938). It is noteworthy that heterochromatin is not only much more susceptible than euchromatin to having structural changes of the gross type induced in it, but also those of the minute type, and that this higher susceptibility to minute rearrangement extends out from the heterochromatin itself to include regions of euchromatin located in its near neighborhood. This is found to be true even when the euchromatic regions in question are normally located far from heterochromatin but have previously, in the given cases, been placed in its neighborhood by means of a prior structural change (Muller, Prokofyeva-Belgovskaya, and Raffel, 1938; Belgovsky and Muller, 1938; Muller, 1938).

Another expression of nonrandomness in the incidence of structural changes of different types lies in the evidence (Bauer, Demerec, and Kaufmann, 1938; Muller, Makki, and Sidky, 1939; Muller, 1940a) indicating that the number of structural changes involving three or more breaks is higher, relative to the number involving two breaks, than would be expected on a chance distribution. This might be due to differences between cells in regard to conditions influencing the likelihood of breakage, and/or differences between them in regard to conditions, such as amount of chromosome movement at the critical stage, influencing the likehood of union of ends derived from different breaks, and/or the fact that, when a broken end does unite with that derived from a different break, it removes the possibility of restitution from the other end derived from the second break and so makes that end more likely than it otherwise would have been to unite with an end derived from a third break. Nevertheless, on account of the spatial limitation, cells in which four or more breaks have undergone rearrangement contain a larger proportion of two by two reciprocal exchanges than if the unions were completely random (Bauer, Demerec, and Kaufmann, 1938).

9. POSITION EFFECTS INDUCED BY STRUCTURAL CHANGES

Only a few paragraphs will be devoted here to the curious consequence of structural change called *position effect*, which has been studied in detail in *Drosophila*, because there is reason to believe that unlike most genetic phenomena studied in the fruit fly, it attains very little expression in most other organisms. In *Drosophila* it has been shown quite conclusively that the type and the intensity of action of a gene in producing its effect upon the organism depends in part upon what genes are in its immediate vicinity in the chromosome thread. Thus, when a gene is

removed by means of a structural change from the genes normally on one side of it and placed near to others, its effect is often changed somewhat, much as if it had undergone a mutation (see Sect. 5). Most often it undergoes more or less inactivation, as happens in many mutations, and in that case the organism with one changed gene and one normal one usually shows the effect chiefly of the normal, the latter then being said to be *dominant* and the altered gene *recessive*. Occasionally, however, the genes changed by position effect are more or less dominant, and in these cases their effect can sometimes be shown to be *neomorphic*, i.e., qualitatively different from that of the normal allele (see Sect. 13). That the change is entirely due to the influence of the neighboring genes upon the given gene is shown, for example, by cases in which the gene in question is returned to its original position, for it is then found to revert to its original mode of functioning (Panshin, 1935; Dubinin and Sidorov, 1935).

Although the spatial range of the effect is minute, it has been shown that not only directly adjacent genes but also those removed by a distance of one or several genes can exert such an influence. For this reason several of the genes located near any point of structural change are likely to have become affected in this way. Since a considerable proportion of all the genes perform some necessary function in the complicated web of biochemical and morphogenetic reactions upon which survival and/or reproduction depend, it is not surprising that, in *Drosophila*, the great majority of structural changes (when received from both parents, and thus allowed to express their recessive effects) result in death prior to maturity or in sterility, and that many of the remainder cause visible abnormalities. This is another reason why, in this organism, relatively few of the structural changes which arise can persist indefinitely in a population.

When genes from a euchromatic region are by means of a structural change placed in the vicinity of a heterochromatic region, or vice versa, the position effects, for some unknown reason, are usually more pronounced, extend over a larger distance (i.e., over a larger number of intervening genes), and express themselves in a peculiar, variegated or mosaic manner (Muller, 1935c, 1938). At the same time the euchromatin comes somewhat to simulate in its cytological appearance and behavior the heterochromatin which has been placed next to it, and the heterochromatin in the same neighborhood becomes more like euchromatin. Under these circumstances, the addition of more heterochromatin to the genetic composition (as when an extra Y chromosome has been inherited) results in both the eu- and heterochromatin in the regions near the point of structural change becoming more like euchromatin cytologically. Along with this goes a lessening of the abnormality of functioning of the genes located near the break in the displaced euchro-

matin, and an increase in the abnormality of functioning of any in the heterochromatin which may have been affected. Subtraction of heterochromatin exerts, as expected, the opposite influence on these position effects.

Interpretations of the mechanism of operation of the position effect are thus far speculative. One hypothesis, proposed by Sturtevant (1925), has been that the effect is produced by changes in the local concentration of gene products, on the assumption that the products of a gene are more concentrated in its neighborhood, and are likely to react to a greater extent with those of another gene when that other gene is nearby and hence has its products also more available for the interaction. Thus the removal or juxtaposition of the latter gene would affect the amount of interaction and hence the expression of any characteristic of the organism which depends on that interaction. According to another hypothesis, proposed by the present writer (1935c, 1941), a gene influences another in *Drosophila* by subjecting it to localized physical forces of stress and strain of the same nature as those which cause genes of like composition to be drawn together in synapsis. But, when the genes are of unlike composition, as would usually be true of neighboring genes, these forces are exerted unequally and asymmetrically on their different parts, so as to mutually influence the shapes of the genes, and with this change in shape would go a change in the type or intensity of the chemical activity of the gene, just as happens when protein molecules are subjected to folding or unfolding.

A distinct case of position effect on genes at two different loci has been found and analyzed by Catcheside (1939, 1947) in *Oenothera blandina*. The effect on both genes here is of the variegated (mosaically expressed) type which in *Drosophila* is, as has been mentioned, characteristically exerted on genes of euchromatin by heterochromatin placed in their neighborhood. Nevertheless, the studies of structurally changed chromosomes in other organisms than *Drosophila* are on the whole conspicuous for the absence of evidence of position effects. If they were nearly as marked and general in occurrence in most organisms as in *Drosophila*, it would have been found in other organisms, as in the latter genus, that the great majority of translocations caused lethality or sterility, or at least some visible abnormality, when received from both parents. It is possible that this is true in the mold *Neurospora*. Yet in mice, as in maize and other forms in which numerous translocations have been worked with, this is certainly not the case ordinarily. The relative uniqueness of *Drosophila* in this respect seems to lend greater plausibility to the view that the cause of the position effect lies in the same influences as those which bring about synapsis. For *Drosophila*, along with most other species of Diptera, differs from most other organisms in having these synaptic influences relatively strongly expressed, not merely at

meiosis in the maturing germ cells but in the ordinary body cells at all times, as shown by the fact that homologous chromosomes even in somatic cells and early germ cells show a strong tendency to lie side by side, with homologous parts in apposition. Hence, if these were the forces which also produced the position effect, it would be expected to be especially pronounced in just this group of organisms.

An important corollary to the relative insignificance of the position effect in most organisms is the inference that the genes must be discrete units or segments of the chromosome, sharply demarcated from one another rather than forming one chemical continuum having no distinction between intra- and intergenic connections. If, as certain authors have speculated, the genes are not discrete entities but only regions exerting given biochemical effects, contained in one long, essentially unsegmented molecule, then it should not be possible freely to break that molecule at practically any point and patch it together again in a different alignment without radically altering the chemical structure and behavior of the parts in the neighborhood of the breaks and new attachments. This is especially to be expected in view of the evidence showing that such a large proportion of the genes is important for life or reproduction. It would therefore seem justified to continue to regard them as separable units, even though, in certain organisms, they or their immediate products do exert influences, extending over a short distance, on each other.

10. INFLUENCE OF STAGE OF CELL AT TIME OF EXPOSURE ON THE CONSEQUENCES OF CHROMOSOME BREAKAGE

The likelihood of production of structural change by a given exposure to radiation, and the type of change produced, depends in considerable measure upon what stage of the cell cycle was treated, i.e., upon the condition of the chromosomes at the time. If a cell at the time of irradiation is (as would usually be the case) in the so-called "metabolic" or "interphase" stage (also miscalled the "resting stage" to distinguish it from the stages of mitosis), then its chromosomes are in a greatly extended, widely dispersed condition, and are usually undergoing only small-range movements. In this situation, even if several different chromosome breaks have been produced, it is very probable that any broken end will, by its Brownian movement, come into contact with the other broken end derived from the same break, thus accomplishing restitution, long before it has a chance to meet an end derived from a different break. And, even in those cases in which it does fail to make a restitutional contact, it is likely not to meet with any of the other broken ends at all until finally, after each of the pieces has reproduced to give two chromatid fragments in preparation for the next mitosis, the adjacent homologous broken ends of each pair of identical twin chromatid pieces

touch and join together, giving an acentric and a dicentric isochromosome, respectively. The dicentric chromosome, pulled to both opposite poles at once, can then be seen (as in Fig. 7-1e) to form a chromatin bridge between the daughter groups of chromosomes at the next anaphase. Thus irradiation during interphase gives relatively few structural changes, and chief among these are chromatin bridges and their complements, lagging acentric fragments, while such types of aberrations as translocations, large deletions, and large inversions are rarely to be found. And, as later and later interphase stages are irradiated, lying nearer and nearer in time to the stage of chromatid formation, these bridges and fragments are produced in ever greater abundance, since less and less time is afforded for restitutional contacts to occur before the homologous broken ends of the adjacent twin pieces become available for union with each other.

If, now, irradiation is carried out during some stage of mitosis, other factors come into operation which hinder restitution and favor the eventual union of ends derived from different breaks, resulting in structural changes. It has long been known that when chromosomes are irradiated while they are in the tightly spiralized, condensed condition characteristic of late prophase, metaphase, anaphase, and early telophase, they do not in most cases appear to be broken at the time, although there are exceptions in some material [see, for instance, A. R. Whiting's (1945) results on meiotic divisions of *Habrobracon*]. However, when the condensing chromosomes reappear at the next mitosis, after the intervening interphase has elapsed, structural changes of varied types are to be found among them in relatively great abundance, as compared with what would have followed irradiation during most of the interphase period. It is evident from this result that the chromosomes when in a condensed condition are in most cases not able to fall apart into fragments, because of some enveloping material, but that their inner threads nevertheless become effectively broken by radiation. Moreover, while in this condensed stage, the broken ends of the threads, although bound close together passively by the material which prevents the pieces from falling apart, are for some chemical reason unable to adhere actively to one another so as to undergo actual restitution, for otherwise their potentiality of later giving rise to structurally changed chromosomes would be lost. It must therefore be concluded that the breaks persist, although invisible, throughout the condensed stage, and that the pieces later, probably in late telophase, tend to fall apart before the ends acquire their mutual adhesiveness. As the chromosomes at about this time begin to enter their relatively unspiralized, extended phase, their parts must undergo much more movement relative to one another than before. Consequently, when the broken ends have finally become adhesive, they are now much less likely to find the other end from which they had been

broken off (leading to restitution) and, conversely, more likely to come into contact with ends derived from other breaks than if they had been broken during interphase, after their positions had become largely stabilized. Thus diverse structural changes finally result from irradiation during mitosis.

The above analysis was, in fact, first arrived at through studies (Muller, 1939b, c, d, 1940a) of the effects of irradiation applied to the chromosomes in mature spermatozoa rather than in mitotic stages. In mature spermatozoa as in mitotic stages the chromosomes are in a highly spiralized, condensed condition. It was possible to prove, by noting the effect of variations in the timing and the dosage of the radiation on the frequency of structural change, that the breaks arising in these spermatozoan chromosomes are all retained as such, without restitution, until after fertilization, when the pieces become able to enter into diverse forms of structural rearrangement. Hence irradiation of spermatozoa, as well as that of cells in and near mitosis, is much more likely to lead to structural changes than is irradiation of ordinary interphase nuclei. Some results have recently been obtained by Ray-Chaudhuri and Sarkar (1952) which they interpret as indicating a similar delay in fusion of broken ends in locust (*Gesonia*) spermatocytes. However, in cytes, unlike spermatozoa, the high degree of separation of the chromosomes allows much less opportunity for contact between different chromosomes or chromosome regions before union occurs. In conformity with this situation it has been found that most of the structural changes induced in *Drosophila* oöcytes are intrachromosomal, and mainly in the class of minute changes (Glass, 1940; Muller, R. M. Valencia, and J. I. Valencia, 1950).

Although there must be some movements of broken ends to enable ends derived from different breaks to meet and result in structural changes, nevertheless these movements are rather restricted in range, even in the production of translocations in chromosomes derived from irradiated spermatozoa. The evidence from neutron irradiation (cited in Chap. 8) shows that two breaks which were produced in close proximity in the spermatozoon give a much better chance of leading to a structural change than do two breaks that were farther apart at the time they were produced. This type of spatial restriction must be far more marked when the irradiation is applied to interphase nuclei, since in these the chromosomes remain, relative to their lengths, much more fixed in position between the times of breakage and union.

The influence of movement in promoting structural change is further shown in Sax's (1942) treatments of *Tradescantia* microspores with two successive irradiations. Chromosomes broken by the earlier irradiation were found to have more breaks that failed to restitute produced by the later irradiation in their centric fragment, which is of course more subject to movement, than in their acentric fragment. Moreover, Sax (1943)

also showed that centrifuging, which of course causes the chromosome parts to be moved about, increases the frequency of structurally changed chromosomes produced by irradiation. So too does sonic vibration (Conger, 1948).

Another limiting factor in the production of structural changes is their time restriction. According to Sax's (1939, 1940) interpretation of his results with *Tradescantia* microspores—an interpretation accepted by most other workers but now disputed by Lane (1951), the broken end of an interphase chromosome has little chance of undergoing union with that derived from another break unless the two breaks have been produced within a rather short time interval of each other, usually of the order of some tens of minutes; otherwise the broken end will in the great majority of cases have come into contact with the complementary end derived from the same break, so as to undergo restitution. This interpretation is based on Sax's finding that a given dose of radiation applied to interphase cells of microspores, if fractionated in time or delivered at a low intensity over a long period, produces fewer structural changes, presumably because of more restitutions, than if delivered in concentrated manner in a few minutes or seconds. A higher temperature during treatment has been shown by Sax and Enzmann (1939) to have an effect on this result similar to that of lengthening the time; this would be because it causes a speeding up of the movements whereby the ends meet each other.[2] Lane (1951) finds, however, in his experiments on *Tradescantia* microspores, that irradiation causes chromosomes to acquire a resistance (which for a considerable period continues to increase) to breakage by later irradiation, and he believes the lesser efficiency of prolonged and of fractionated radiation (and presumably of that applied at a higher temperature) to be entirely explained in this way, without assuming that union can occur during interphase, in this material. Nevertheless, the fact that in many experiments the structural changes produced by irradiating long before mitosis involve whole chromosomes rather than chromatids shows that in these cases union occurred before effective chromosome reproduction into chromatids occurred, i.e., at some time during interphase, when the chromosomes were in an extended condition.

In recapitulation, it may be observed that the far lower frequency of both viable (eucentric) and inviable (aneucentric) structural changes induced by irradiation of ordinary interphase stages than by that of spermatozoan and mitotic stages must in considerable measure be the result of the greater spatial limitation on union of broken ends when the

[2] It will be noted in Chap. 8, however, that there are one or more other ways in which temperature can influence the frequency of production of structural changes, quite apart from that discussed above. These other types of temperature influence may operate even when condensed chromosomes are irradiated.

breaks are produced in interphases. If, as is usually held to be the case, there is also a greater time limitation during most interphases, this would constitute a second circumstance working in the same direction. Working in the same direction also, as far as the formation of inviable isochromosomes is concerned, is the third circumstance, that during most of the interphase stage the chromosome threads behave, in regard to breakage by radiation, as though they were single, and also manage to have their broken ends unite before they become effectively double, while for chromosomes irradiated during or shortly before entering a condensed stage one or both of these conditions usually fails to hold, and dicentric and acentric isochromosomes can be formed in consequence. It is not yet known whether these three circumstances are the only ones which lie at the basis of the special resistance of most interphases, as compared with premitotic, mitotic, and spermatozoan stages, to having structural changes produced in them, or whether in addition the chromosomes are actually less breakable during interphase, but there is at present no way of measuring their breakability, uncomplicated by phenomena involving union of broken ends.

The greater vulnerability of spermatozoan chromosomes to structural change constitutes the chief reason that spermatozoa have usually been chosen for irradiation in experiments in which the production of such changes was desired. Conversely, this is also the reason that it is desirable, for the production of offspring as free of structural chromosome changes as possible, to allow a sufficient interval (in mammals, of some months) to elapse after irradiation of the male before reproduction is allowed. For in this way the germ cells which were in immature spermatogonial interphase stages at the time of irradiation have been given time to replace those which were irradiated while in the mature condition. As for the female, it has been noted previously that the higher degree of dispersion of the nonhomologous chromosomes of oöcytes results, in *Drosophila*, in fewer translocations being produced by irradiation of oöcytes than of spermatozoa. In mammals, however, some translocations have been induced by irradiation of oöcytes, although their frequency remains to be better determined. It is not known how much, if any, the frequency of these would be reduced by increasing the interval between irradiation of the female and reproduction; since in mammals the oöcyte stage persists for a very long period, it may be that the stage sensitive to the production of structural changes lasts much longer in female than in male mammals.

Finally, the above principles appear to provide the chief reason that there is so much more damage caused by radiation to somatic tissues in which cell divisions are abundant than in those in which they are rare or absent. Additional reasons for the greater damage to tissues with more

frequent mitosis are the facts (1) that radiation also induces aneuploidy by nondisjunction and by lagging of whole chromosomes when it is applied within a limited period (some days) before mitosis (see Sect. 3-2) and (2) that unless a cell undergoes at least one mitosis subsequent to its irradiation, any structural changes that may have been induced in it must fail to result in either chromosome bridges or aneuploidy of any kind, and hence must remain comparatively innocuous.

These radiogenetic considerations, then, furnish an explanation of the high correlation between the amount of proliferative activity of an organism, organ, tissue, or type of cell, and its susceptibility to being damaged by radiation or other agents (such as mustards) that cause structural chromosome changes. It is only to be expected, on this basis, that the younger an individual is, all the way down to the stages of early cleavage, the greater is the damage caused by a given dose of radiation; that those parts are most affected, and most checked in growth, which grow more actively; that regeneration and wound healing tend to be inhibited; and that in the adult the tissues selectively affected are germinal tissues, blood-forming tissues, the epidermis and its derivatives, mesodermal parts that require cellular replacement, proliferative endothelia and endodermal epithelia, and malignant growths of all kinds.

On the other hand, it must not be assumed that, in general, developmental abnormalities that result from irradiation of the embryo are always expressions of chromosomal damage. Processes of morphogenesis can be affected during their sensitive stages by the influence of radiation just as by other toxic influences, such as high temperature or certain chemicals, so as to shunt them into some abnormal direction, without any significant genetic change having been induced in the nuclei of the cells concerned. That this is the case in irradiated *Drosophila* embryos was found by Lamy and Muller (1939) by comparison of the effects on diploids and polyploids: these failed to show the consistent differences to have been expected between them if the effects had had a genetic basis. That the radiation damage to developing individuals which causes their death is, however, in some cases due to genetic changes was shown by the contrary results of A. R. Whiting and Bostian (1931) and of Clark and Kelly (1950), who used similar techniques with immature stages of the wasp *Habrobracon* which differed from one another in the number of their contained chromosome sets. In the latter material the damage was inversely correlated with chromosome number in just the manner to be expected of the effects of induced chromosomal changes. In cases in which the question is not concerned primarily with the determination of the type of morphogenetic processes, but rather with the capacity of tissues for proliferation and survival, we are on firmer ground in invoking chromosome change as the usual means by which ionizing radiation produces its long-term damage.

11. MANNER OF INCIDENCE OF RADIATION-INDUCED AND SPONTANEOUS MUTATIONS OF GENES

The genetic effect of ionizing radiation that is most important in its long-term consequences for an exposed population is the production of *gene mutations*, i.e., permanent, heritable changes in individual genes. This is also the genetic effect of radiation that is of greatest theoretical significance. Nevertheless, gene mutations are by no means effects peculiar to radiation, for mutations of sensibly the same types are continually arising "spontaneously" on a widespread scale, without the application of radiation or any other special treatment. Moreover, they can also be influenced greatly in their frequency of occurrence by various conditions and agents other than radiation. Radiation is, however, the first highly effective means that was discovered for producing gene mutations in quantity, as was shown in experiments on X-rayed *Drosophila* carried out by the present writer in 1926–27 (Muller, 1927, 1928a, b, d) and soon afterward confirmed in the same material by Weinstein (1928). Moreover, it still remains in the first rank of agents having this effect. The effect is produced by ionizing radiation of varied kinds. The first decisive results with ionizing radiations other than X rays were reported by Hanson (1928, *et seq.*), working in consultation with Muller, for β and γ rays; by Ward (1935), working under Altenburg's direction, for α rays; and by Nagai and Locher (1937), working under Altenburg's direction, for neutrons. The effect is also produced by ultraviolet, as was first shown by Altenburg (1930), after earlier negative results in 1928.

Work on the production of mutations by X rays in higher plant material was being carried out by Stadler and by Goodspeed and Olson independently of and simultaneously with the earliest successful experiments along these lines in *Drosophila* but, on account of the longer time necessary for the growth of the plants, the definitive generations were not obtained until 1928 (Goodspeed and Olson, 1928; Stadler, 1928a, b, 1930, *et seq.*). In Stadler's work, recessive "point mutations" (a general term for mutations showing regular Mendelian inheritance and not connected with gross chromosome changes) were produced both in maize and in barley. As will be noted in more detail in Sect. 16, however, Stadler has been inclined to interpret most or all of these point mutations produced by X rays in his material as minute deficiencies rather than as true gene mutations, while the mutations which he much later obtained by ultraviolet in maize show more agreement in their characteristics with the spontaneous changes generally regarded as gene mutations. In *Drosophila*, on the other hand, it was evident practically from the start, from the series of varied alleles, including mutations in the reverse direction, arising at given loci, that gene mutations were being produced by the ionizing radiation. In Goodspeed's experiments the main changes

observed proved to be gross chromosomal aberrations, and it remained possible to apply this interpretation even to those changes which were inherited in a more or less Mendelian manner.

Although the works mentioned were the first to give definite evidence of the abundant production by radiation of point mutations and structural chromosome changes that were transmitted to subsequent generations, they had of course been led up to by a long succession of experiments on the effects of ionizing radiation on the hereditary material. Thus the production of abnormal and moribund embryos from irradiated sperm in amphibia, mammals, fish, and echinoderms, reported in the years 1907 to 1913 (see Chap. 8, Sect. 14), had been generally regarded by those conducting the experiments as evidence of damage to genetic material in the chromosomes. It was, however, much longer before clear-cut results capable of genetic interpretation could be obtained.

It is true that certain suggestive results were reported. Among these were alterations in the manner of growth and nutritional requirements of mold colonies, produced by radiation in work of Dauphin (1904); somatic abnormalities, not transmitted to the next generation, which were found by Gager (1908a, b) in *Oenothera* derived from germ cells treated with radium; and two peculiar variations, Beaded and Truncate wings, of unclear mode of inheritance, found by Morgan (1911) in descendants of radium-treated *Drosophila*. Loeb and Bancroft (1911) also reported finding some mutations in *Drosophila* after radium treatment, but their manner of appearance, in both treated and control lots, led other *Drosophila* workers to infer that the genes concerned had been present heterozygously in the original stocks. Similar doubts seemed justified in the case of the mutations found by Guyénot (1914) after ultraviolet treatment of *Drosophila*. On the other hand, in a number of experiments on other material, negative results of radiation treatments on the characteristics of later generations were reported, but these could not be regarded as definitive either.

The attack had been renewed in the third decade of this century. For example, Unterberger (1922) reported obtaining butterflies of a size which diminished from generation to generation among the descendants of irradiated but not among those of nonirradiated individuals, yet results of this kind (which have not been obtained by others) appear, on genetic grounds, to be very dubious. Again, Little and Bagg (1923), on inbreeding descendants of mice which had been irradiated, did find four undoubted mutations of different kinds, but their controls, which were only half as numerous, showed two mutations, one of them identical with one of those in the treated series. (In the light of present knowledge, the dose used by them was so small that no statistically perceptible genetic effects would be expected from it.) Nadson and Philippov (1925), on the other hand, certainly obtained inherited abnormalities

with a much higher frequency in the descendants of irradiated than of nonirradiated molds, but here genetic analyses were wanting, and the results might have been attributed to such phenomena as somatic segregation or nondisjunction. Gager and Blakeslee (1927), on going over a body of data on descendants of radium-treated *Datura*, obtained before 1922, and comparing it with control data obtained since that time, were able to show that many so-called "chromosome mutants" had indeed been produced by the radiation, but most of these were types having an entire but normal extra chromosome, brought about by nondisjunction, an already known effect of radiation (see Sect. 3-2). There was however one case of a structurally changed chromosome, and two cases of recessive visible mutations.

The reason that other investigations, carried out in the later 1920's, succeeded in obtaining more conclusive results than all these lay in the great developments which both genetic technique and genetic theory, based on studies of nonirradiated material, had by that time undergone. These made discriminations between mutagenesis, on the one hand, and both environmentally induced "modifications" and genetic effects of inbreeding, on the other hand, more precise, and also made the analyses into different classes of heritable changes more informative.

In order to view the more definitive work on the production of changes in genes by means of radiation in its proper perspective, the results of the prior work on gene mutation and its converse, gene stability, in the absence of artificially applied radiation, should be briefly reviewed here. Considerable evidence had accumulated—for instance, in the work of Johannsen (1909) on beans and of Muller and Altenburg (1919) on *Drosophila*—that genes are ordinarily very stable. Although they are capable of undergoing "spontaneous" permanent changes these changes are, for any given gene, rare, sudden, and discrete, causing the gene to pass from one stable state to another. The stability of each gene is such as to preclude its undergoing frequent small fluctuations of an inheritable nature, as assumed on the view of "continuous variation." If these frequent small changes occurred they would tend to accumulate, so as to result in changes of perceptible size, and these perceptible changes of any gene, when classified according to size and number, would then be grouped in a "probability distribution," and would make possible the progressive success of continuous selection of a given gene in a given direction. This is contrary to the results of exact observations on gene changes (Muller, 1918, 1920; Altenburg and Muller, 1920).

The finding that an individual gene undergoes a definite transformation when it mutates spontaneously, and that in the intervals between mutations it maintains a fixed composition, was difficult to interpret except by the view that each gene, like a molecule, has a distinctive chemical struc-

ture, and that its mutation represents a change in that structure which, like all chemical changes, is subject to the all-or-none rule of quantum events. The same principle of sudden discrete change, preceded and followed by stability, was then found to hold for the mutations induced by radiation. This result, obtained with an agent known to cause individual quantum changes of atoms and molecules, tended to confirm the interpretation of mutation as involving definite chemical recombination. In a sense, however, this is almost stating the case backwards, since it was chiefly these and related considerations, given below, which had led the writer to the testing out of the possibility that ionizing radiation produces mutations.

The view that a gene mutation represents a definite chemical change in the composition of the gene does not imply that, as in a reaction in a test tube, a definite product could be produced to order by adding a certain reagent or arranging conditions in a certain way. Mutation does not occur on a molar scale but, on the contrary, each mutation represents one submicroscopic transformation, instigated by a physicochemical situation, the minute localization of which, in the effective neighborhood of this gene or that gene, must depend upon the many chance factors of what Troland (1917) aptly called "the molecular chaos." That this was the case was at that time already indicated by the fact that spontaneous mutations appeared to happen largely at random, without reference to the type of environment. Even under the most constant conditions of living obtainable, mutations of the most diverse kinds continued to arise in an apparently sporadic fashion, while, conversely, changing the environment had no discernible effect in causing mutations of given types to be found. Moreover, when a mutation arose, only one gene in the given chromosome, and indeed in the nucleus, underwent change at a time.

Especially telling was the evidence, the significance of which was pointed out by the present writer (1920, 1922), showing that when mutation occurs in a gene in a given chromosome of a diploid cell, the homologous gene of identical composition, which in *Drosophila* lies in a corresponding position in the homologous chromosome at a distance from the first gene, usually, of only a fraction of a micron (because of the close pairing of the chromosomes even in ordinary interphase stages in *Drosophila*), fails to undergo any change at all. Thus the mutation could not have been due to the presence, in molar amount in the cell, of some special chemical, of such composition as to have a pronounced proclivity for reacting with that particular type of gene rather than with genes of other types. For in that case there would have been a tendency for both identical genes to have been attacked at once. Instead, the decision that this gene rather than another one underwent mutation on a given occasion evidently depended upon the chance ultramicroscopic distribution of

pointlike disturbances, of such a nature as to be capable of changing practically any gene. Now this is just the mode of operation of radiation when it is applied to a complex mixture of organic substances in aqueous solution.

It was not so surprising, then, that radiation should prove to be effective in the causation of gene mutations. This being the case, it was also quite in line with expectation that the radiation mutations of genes, like the spontaneous ones, should be found to occur according to a sporadic, pointwise, essentially fortuitous pattern of incidence. This resemblance extended even to the finding that the gene mutations produced by radiation in diploid cells involved only one of any two identical genes present (Muller, 1928d).

A further parallelism lay in the fact that, as had already been shown for the spontaneous gene mutations (Bridges, 1919; Muller, 1920, 1928c; Muller and Altenburg, 1921), the occurrence of radiation mutations was found not to be confined to any given type of cell or period of development, since analysis of experiments designed to test these questions showed the mutations to be produced in either mature or immature stages of the individual, in females or males, and in germinal or, as first shown by Patterson (1929), somatic cells. It is true, however, that their frequencies of production in some of these different situations did appear somewhat different—a matter to be taken up in more detail in Chap. 8. Now the mutations that were produced in early (primordial or gonial) germ cells necessarily resulted in a whole group of offspring carrying the same mutant gene (Harris, 1929). Similarly, those produced in somatic cells which later proliferated resulted in a whole patch of tissue or part of the body having the mutant gene.

It should be observed that these cases of the derivation of a visibly abnormal portion of the body from a single mutant cell illustrate by analogy the gene-mutation interpretation of the causation of some malignant growths. If any of these somatic mutations should chance to be of such a nature as, singly or (more likely) in combination, to result in continued unregulated proliferation, a neoplasm or other malignancy would thereby have been induced. In view of the great number of different genes in every cell, the exceedingly diversified character of different gene mutations, and the vast number of cells in the adult body which are still capable of undergoing some proliferation, it would be strange indeed if a gene mutation, or, eventually, a combination of mutations, did not sometimes arise, which conferred on the cell containing it the property of proliferating to an unlimited extent, even in the face of the checks to growth which are continually provided by the regulative morphogenetic influences of the surrounding normal tissues.

It is not to be expected that additions or subtractions of whole chromosomes or even of gross parts of chromosomes would, as postulated in the

pioneer suggestions of Boveri (1914) when he proposed the somatic mutation view in its first crude form, provide any material subtly and delicately enough differentiated to succeed in carrying out such growth in the face of the competition and opposition of the normal tissues. But mutations and perhaps also, sometimes, minute losses of genes—in any case what are called "point mutations" (a term somewhat broader than "gene mutations"—see p. 389) should afford a sufficient range and specificity of changes to include an occasional alteration having such an effect. As one line of evidence that such is the case, it is to be observed that the point-wise, sporadic manner of origination of malignant developments finds a parallel in the manner of occurrence of mutations. Moreover, the fact that the same agent, namely, radiation (either ionizing or ultraviolet), which produces mutations is also effective in producing these growths adds to the plausibility of the interpretation. A further discussion of this matter will be reserved for Sect. 19.

12. RELATIVE FREQUENCIES OF DIFFERENT TYPES OF CHARACTER CHANGE CAUSED BY RADIATION-INDUCED AND SPONTANEOUS MUTATIONS

Examination of the types of effect produced by gene mutations shows that in *Drosophila* those radiation mutations which have a visible morphological expression resemble in their general distribution of types the ones which have arisen spontaneously. This does not necessarily mean that the relative frequencies of mutation for different genes—still less, those of different kinds of mutation (to alleles of different kinds) for the same gene—are identical for radiation mutations and spontaneous ones. Spontaneous mutations are too rare to have allowed a reliable frequency distribution of this kind to have been made for them, except in a few special cases. However, experience has indicated that any type of gene mutation which has been found to arise spontaneously can also, when an intensive search is made, be found after the application of ionizing radiation, and probably also after ultraviolet treatment, and that the converse proposition holds likewise. Moreover, the kinds of morphological effects do occur in similar relative frequencies. There are, for instance, in both the radiation and the spontaneous series in *Drosophila*, very many mutations, in any one of numerous different genes, which give a minute bristle effect, and rather many that give roughened eyes or wings held apart, while on the other hand mutations of so-called "achaete" appearance, which cause an absence of bristles that is largely restricted to the middle of the back, are exceedingly rare in both series. Again, studies by the writer and more especially by Timoféef-Ressovsky (1937) have indicated that the ratio of mutations so detrimental as to be practically certain of killing the individual before its maturity—those designated as *lethals*—to

those which permit considerable survival but result in some abnormality readily visible to the trained observer—the *visibles*—is about the same, namely, in the neighborhood of 7 or 8 to 1, both for spontaneous mutations and those induced by ionizing radiation.

Although (as was stated on p. 391) the different mutations of any single gene fail to be distributed, as regards the frequencies of those giving different amounts of effect, in anything like a "normal curve"—inasmuch as those of more extreme effect are often commoner than those of lesser effect—nevertheless when a given *character* (e.g., eye color), instead of a single gene, is examined, and mutations in all the numerous genes which affect that character in any degree are taken into consideration, it is found that the mutations affecting that character to a lesser degree are more frequent than those of greater degree (Muller, 1923). This is not surprising in view of the complicated net of biochemical reactions that underlie both the processes of general metabolism, those of morphogeny, and those of special physiology, since it is to be expected that there would be fewer genes with a strong, specialized effect on the development of any given character than those which (having been specialized primarily in relation to other characters, which are likely to be invisible) influenced the character in question merely incidentally and slightly. There is indeed evidence that still more frequent than the mutations with slight yet appreciable effect are those the effect of which, on any visible character, is below the threshold of detection by ordinary means (Altenburg and Muller, 1920). This general principle holds both for spontaneous and radiation mutations.

One of the most all-embracing, generalized "characters" capable of being observed and measured is the *viability*, i.e., the ability to survive until some given point in the life cycle has been reached; in *Drosophila* this is usually taken, for convenience, as the beginning of the reproductive period. The measure of viability of a mutant then is the frequency with which individuals of the given type survive to maturity, as compared with (divided by) that with which nonmutant individuals do so. Those mutant genes which allow no individuals at all to reach this stage are the ones designated as (complete) lethals; those which have between 0 and 10 per cent of the normal survival rate are for convenience distinguished as "semilethals" or (a better term for them) *sublethals;* while those with over 10 per cent but less than 100 per cent of normal viability are termed *detrimentals*. The great majority of mutants (whether spontaneous or radiation induced) which cause any kind of externally visible morphological abnormality are found to be in some degree detrimental, and, in a general but very imperfect way, there is a tendency for a greater degree of visible abnormality to be associated with a greater amount of detriment (lower viability). This prevailingly detrimental nature of mutations, of whatever origin, is undoubtedly a consequence of the fact (also

mentioned in Sect. 1) that in a living thing, as in any complicated organization the parts of which have been selected so as to interact nicely for the accomplishment of a given difficult end result (this result in the case of all living things being survival and reproduction), any change of a part, initiated in a random way (i.e., without foresight or selection for that end), brings about in the vast majority of cases a less efficient functioning of the system (Muller, 1918, 1923).

We have already seen (p. 395) that the lethals greatly outnumber the visibles. It had long been suspected, however, that there are many more detrimentals without visible effects than lethals, in line with the idea that for viability, as for other characters, "small" mutations (i.e., those with small effects) are more numerous than "large" ones. Because of the difficulty of detecting such invisible detrimentals, their frequency among spontaneous mutations has not yet been investigated. In the case of radiation mutations, however, the matter has been studied. The results obtained in two independent, simultaneous series of investigations, one by Timoféef-Ressovsky (1935a) and the other by Kerkis working under the direction of Muller (Muller, 1934; Kerkis, 1935, 1938), agree in showing that in the X chromosome of *Drosophila* invisible detrimentals are induced with some 3 to 5 times the frequency of the combined class of lethals and sublethals. The ratio may indeed be considerably higher than this, since the technique was hardly refined enough for the detection of detrimentals with a viability greater than some 85 per cent of the normal. Other studies have shown that "invisible" mutants causing sterility or lowered fertility of some degree also form a very large group. This group, however, overlaps, to an extent not yet well investigated, that of the detrimental mutations.

It is evident from this discussion that if mutations could be arranged in order of the conspicuousness of their effects on the organism, so as to form a kind of spectrum, there would be a comparatively narrow "visible" part of this spectrum, with a much larger region to "the right" of it, comprising the lethals, with effects so drastic as to remove the individuals from our view before maturity, and a still larger region to "the left," comprising the genes with invisible effects. This principle, first encountered in the case of spontaneous gene mutations (Muller, 1923), has since then been shown, through the much more detailed studies cited, to hold also for those induced by ionizing radiation.

13. EFFECTS OF CHANGING THE RELATIVE QUANTITIES (DOSAGE) OF GENES

All the classes of mutations mentioned in the preceding section, distinguished according to their kind and degree of expression, i.e., according to the set of observable characteristics or *phenotype* of the individuals

containing them, are in a sense arbitrarily defined for our convenience and depend largely upon the techniques used in detecting the mutations. They intergrade and overlap each other widely. Moreover, there is no reason for assuming that these classes correspond to any consistent differences in the kinds of changes that took place in the genes when the mutations occurred, or in the kinds of biochemical influence of the mutant genes in the different classes, as compared with those of their normal alleles. There is, however, a method by which some light may be thrown on the latter question. This is by comparing the effects produced, i.e., the phenotype, in individuals known to have different "doses" (numbers of representatives) of given mutant genes, and in those having different doses of their normal alleles. This is made possible by the fact that by irradiation, taken together with suitable genetic techniques, structurally changed chromosomes or sets of chromosomes can be found in which a small chromosome region containing the given gene has been lost by deletion, or, in some cases, by crossing over or recombination between chromosomes of slightly different structure. Conversely, individuals can be obtained which have, in addition to the doses of the gene expected in a diploid, one or more extra chromosome sections, of small size, containing the given gene.

Studies of this kind by the present writer (1932b, 1950a) have indicated that in the majority of cases when the dosage of a mutant gene (no matter whether of spontaneous origin or induced by radiation) is decreased from two to one, in the absence of any normal allele of the given gene, the abnormality becomes intensified; while, vice versa, when the dosage is increased from two to three, the phenotype becomes more nearly normal. Inasmuch as a higher dosage of such a mutant gene, causing a greater concentration of its biochemical products in the cell, results in an effect more nearly like that of the normal gene, it must be concluded that the mutant gene has a biochemical action, the effect of which is similar to but less marked than that of the normal gene. A mutant gene of this type is therefore called a *hypomorph*. This result was anticipated, on the ground that the normal gene represents a highly organized system, resulting from the selective survival and accumulation of a long series of changes that were conducive to producing the given advantageous effect, and that in consequence most new changes occurring in it without previous selection or foresight would result in its lesser effectiveness in the carrying out of its specialized functions (Muller, 1923). This situation is analogous to that already discussed (p. 396), which exists on the level of the system of genes as a whole, whereby most changes cause a lesser ability to survive and reproduce.

Although the hypomorphs appear to constitute the majority, not all mutant genes are in this category. There is also a fairly common group called *amorphs*, in which there is no longer any trace of the normal effect

in question. This is to be concluded from the fact that a change in dosage of the mutant gene in such a case does not influence the degree of expression of the given character. In many of these cases hypomorphic alleles of the gene have been found as well, which show a similar but less marked difference from the normal and respond to dosage increases by causing an approach to the normal phenotype. In other cases, where hypomorphic alleles have not been found, the fact that the amorph stands at the zero level on the scale of activity of the type responsible for the differences studied can be deduced from the fact that reduction in dose of the normal gene is manifested by a character change similar in kind, but usually much smaller in degree, than that found in the presence of the amorph.

There appears to be a much rarer type, termed an *antimorph*, which has an action opposite in direction to that of the normal gene, in that an increase in dosage of the mutant gene, when the normal gene is not present, causes a greater departure from the normal phenotype. Some cases previously considered as antimorphs probably belong in other categories, however, since at first it was not realized that a mutant allele could, without being an antimorph, actively compete with the normal in the determination of the phenotype in individuals having both genes (see p. 404).

There is no doubt that changes in the *hypermorphic* direction can occur as well, although, for reasons to be given in Sect. 14, such mutations of normal genes would usually be very difficult to detect. Certainly differences, ordinarily subliminal in their effect on the phenotype, have been found between the normal alleles of different populations, of such a nature as to show that one of these alleles was hypermorphic in relation to the other. From the standpoint of the more effective gene on the other hand the less effective one (although "normal" for its population) would be hypomorphic. Although in these cases one could not know which one represented more nearly the ancestral condition the answer to this question in any given case is relatively unimportant in view of the evidence, reviewed in the following paragraph, that mutations (both spontaneous and radiation-induced) can take place in each of two opposite directions.

Among the types of change that can be brought about by gene mutation are transformations of a mutant gene, usually of a hypermorphic nature, which cause it to give a phenotype more nearly like, or even in some cases sensibly identical with, that produced by the normal gene from which the mutant gene had been derived. It is in line with the conception, previously presented, of the greater likelihood of a mutation causing a degradation rather than an improvement or increase in gene functioning, that these *reverse* or *normad* mutations arise, in the case of most genes, with much lower frequency than the so-called *direct* or

abnormad mutations (those to a less normal phenotype). Yet the fact that they can occur at all shows that not all mutations are of the nature of losses. Moreover, the finding that even amorphs can give reverse mutations to or toward normal (sometimes, however, requiring two steps for the actual attainment of normality) shows that, despite their having lost the ability of producing a given biochemical reaction, the genes themselves are in these cases still there, and retain much of their original structure.

Of course the mere fact that a mutation has caused a change in a given mutant character to or toward normal is not in itself evidence that a true reverse mutation of the given mutant gene has occurred. For a mutation in a second, quite different gene sometimes has a so-called "suppressor" effect, that is, an effect antagonistic to that of the mutant gene primarily under consideration, so as to cause the phenotype of an individual having both the original mutant gene and also the second or suppressor gene to be more nearly normal than that of an individual with just the first mutant gene. Therefore all suspected reverse mutations must be subjected to genetic analysis before they can be known definitely to be true reverses. Definite reverse gene mutations, proved to be such by genetic analysis, have been obtained by ionizing radiation for a considerable number of genes of *Drosophila* (Muller, 1928d; Patterson and Muller, 1930; Timoféeff-Ressovsky, 1929, 1931b, 1932, 1933a, b). They have also been obtained in the mold *Neurospora* (Giles, 1952) by application of both ionizing radiation and ultraviolet, and nutritional deficiencies have thereby been restored. It is still a question, however, whether those produced by ionizing radiation in *Neurospora* involve actual gene mutations or structural changes; it seems not unlikely that some of them may involve one of these phenomena and some the other. Moreover, it cannot justifiably be assumed that even a "true" reverse gene mutation necessarily restores the precise chemical configuration of the original normal gene.

Probably the most interesting class disclosed by the dosage studies is that of *neomorphs*, already mentioned in Sect. 9. This class, like the others, is found among both spontaneous and radiation mutations. An increase in dosage of the mutant gene in this case increases the departure of the phenotype from normal. Yet such a mutant gene does not cause a reaction opposite or antagonistic to that of the normal allele, or one that competes with the latter, since in these cases a change in the dosage of the normal gene itself exerts no influence on the given effect. Thus the *neomorph* is the cause of some reaction of a different nature from that mediated by the normal gene, a reaction which in this sense is "new" to the organism. So far, all the neomorphs studied appear to have been cases in which the functioning of the normal gene had been altered by the position effect of a structural chromosome change, rather than by a

gene mutation proper. However, it seems reasonable to infer that practically any types of alterations in gene functioning that can be brought about by a position effect could also, on occasion, be accomplished by some kind of change within the gene itself. In fact, if neomorphs had not been able to arise by gene mutation, genes could hardly have become differentiated from one another by mutation, in the long course of evolution, in such wise as to give rise to ever more complicated organisms, incorporating new types of biochemical reactions.

It must not be supposed that the above classes are absolute. Some mutant genes have a complicated series of effects, some of which belong more nearly in one category and others in another. Moreover, although a given gene may undergo different mutations, the effects of which appear to differ only in degree, in not a few cases (both of spontaneous and radiation origin) the effects of the different mutations are qualitatively unlike. Usually a gene, by its mutations, can cause a change in more than one character, and in some of these cases not all the effects run parallel, as one allele may show character *a* more affected than *b* while another allele shows *b* more affected than *a*. These and related facts give evidence of the complexity of the individual gene and of the multiplicity of the types of change that it can undergo.

Although differences in the dosage of hypomorphic mutant genes for visible characters usually occasion marked differences in the phenotype (the higher doses being more nearly normal in expression), changes in the dosage of the corresponding normal genes usually have extremely little or, most often, no effect at all that is detectable by ordinary inspection. This is connected with the fact that the phenotypes of hypomorphic mutants also show a tendency to be readily influenced by differences in the environmental conditions existing during development and by differences (caused by mutation) in numerous other genes, which do not perceptibly affect the given character when the normal allele of the hypomorph is present. In other words, the effect of the more weakly acting gene, the hypomorph, is more variable than that of the normal gene.

These results are understandable, as pointed out independently by Plunkett (1932) and by the present writer (1932b, 1935b, 1950a), when it is realized that these dosage studies show that increase in gene dosage, i.e., in gene concentration, or in its equivalent, gene activity, is usually accompanied in its early stages by an approximately proportionate increase in the phenotypic effect, but that as larger and larger doses or greater gene activities come into play the effect increases ever more slowly. That is, the curve relating the effect (as ordinate) to the gene dosage or activity (as abscissa) (see Fig. 7-6) rises at first in a straight line from the base line at the origin, but as it proceeds to the right its slope gradually decreases, tending toward the horizontal as a kind of saturation level of effect is approached. This falling off in efficiency is a

result to be expected when the amount of any reagent is increased which has a limited amount of material to work in and to exert its effect upon. Moreover, since any of the numerous environmental conditions or genetic agencies ("modifying genes") which can influence the activity or effectiveness of the given gene, or gene-product, in producing its end result or phenotype will have an effect like that of changing the dosage or activity of the gene, it follows that such agencies, when acting on developing individuals that have a low activity of the given gene, corresponding to the left-hand, rapidly ascending region of the curve, will by sliding the effectiveness backward or forward also slide the character up or down correspondingly. Thereby its high variability at such levels is accounted for. Conversely, in the right-hand, more nearly level, region of the curve, the same influences, even though acting as strongly as before on

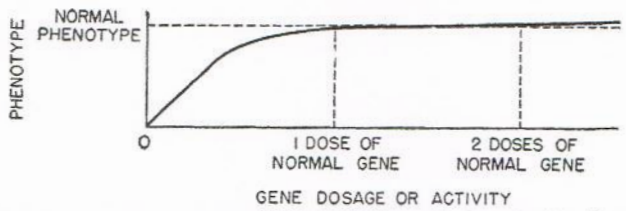

FIG. 7-6. Relation between gene dosage or activity and phenotypic effect in the case of hypomorphic mutants.

the biochemical processes concerned, will succeed in causing little or no perceptible vertical deviation, representing alterations of the phenotype.

Undoubtedly the normal genes were established through a long process of natural selection of appropriate hypermorphs, in consequence of the very fact that, because of the mechanism just discussed, such intense activity gave the development of any given character that greater stability which was advantageous to the organism and the species. That the character, as normally developed, is at an optimum level in terms of advantage for the organism, is readily demonstrated by tests showing the lower ability of the mutant types to perform the functions in question. The same conclusion is also supported by other cogent evidence, derived from a phenomenon called "dosage compensation" (Muller, 1932b, 1950a), which is, however, too intricate to be explained here. In view of the character being at its optimum level, it is also advantageous to have its development proceed as reliably as possible for attaining precisely this most advantageous degree of expression in all individuals. This situation, then, inevitably led to the selection of genes of sufficient activity to operate in the near-saturation region of the gene activity-character curve, for thereby the required stability would be attained as nearly as possible.

The actual phenotypic level at which the curve in question stands when

at this near-saturation level is another matter, for it would have been subject to regulation by mutations in accessory genes as well as in the "primary" gene. In this way the level could be prevented from being set at an unduly high mark, despite the fact that the primary gene was at near-saturation activity. The details of the phenomenon of "dosage compensation," alluded to above, have made it clear that this adjustment of the near-saturation level of the developmental reaction was as important as its attainment of stability.

14. DOMINANCE

An important consequence of the high activity attained by the normal gene, and of the strong similarity resulting therefrom between individuals with different doses of the normal gene, is the fact that individuals having a normal gene from one of their parents and a hypomorphic or amorphic mutant allele from their other parent closely resemble, or are sensibly identical with, those having a normal gene from both parents. That is, the normal gene tends to be *dominant*, the mutant gene *recessive*. This is to be expected if the effects of the two different alleles in an individual having one normal and one hypomorphic or amorphic mutant gene tend to be cumulative, like those of added doses. For in such an individual the character in question would be developed at least as strongly as in an individual with but one dose of the normal gene and with a deficiency in the homologous chromosome, and, as we have seen above, this level of the character is practically as high as in the individual with two doses of the normal gene. Some mutant genes, to be sure, exercise a somewhat competitive action (see p. 404) and thus cause the effect, in an individual having one such gene and one normal gene, to be less than that to be expected from a simple addition of the two respective doses, but this reduction is seldom enough to cause a perceptible deviation from the normal phenotype.

A few more definitions are in order before proceeding further. An individual that has received from its parents two genes of identical type, lying in a given position or locus in a given pair of homologous chromosomes, is said to be *homozygous*, or a *homozygote*, with respect to the genes at that locus, while an individual which has received two different alleles of that gene is termed *heterozygous*, or a *heterozygote*, for that locus. *Dominance*, in the sense in which the word is sometimes used, implies only that the phenotype of the heterozygote does not, in the case of a given pair of alleles, stand midway between those of the two contrasted homozygotes. In other words, some degree of dominance may be said to exist whenever the heterozygote resembles one of the two homozygous types distinctly more than it does the other one, and the gene for the first type is then termed the dominant one.

In practice, the dominance of one allele over the other appears in most cases to be virtually complete, if a superficial inspection only is made. In such cases the heterozygote cannot be distinguished from the dominant homozygote by ordinary means. Moreover, whether or not the dominance is as strong as this, the normal allele is, with but rare exceptions, the dominant one in the above sense of the term. For although the mutant is sometimes loosely termed dominant in any case in which the heterozygote is readily distinguishable from the homozygous normal, nevertheless it is usually found even in such a case that the heterozygote is still more different from the homozygous mutant. At least this is true for hypomorphic and amorphic mutants.

Despite the apparently complete dominance of the normal gene in the great majority of cases, a more searching investigation, e.g., by measurement of numerous individuals and statistical analysis of the data, or by the use of biochemical techniques or studies on rates of survival, usually shows that the heterozygote is after all not quite the same phenotypically as the homozygous normal type. For example, studies on lethal and sublethal mutations in *Drosophila* (Stern *et al.*, 1948, 1951, 1952; Muller, 1950b, c) indicate a dominance of the normal gene of something in the neighborhood of 96 per cent, leaving about 4 per cent of expression to the mutant in the heterozygote, on the average; i.e., the viability of such heterozygotes is only about 96 per cent as great as that of homozygous normals. This is also expressed by saying (with a slight alteration in the use of the term dominance from that of our former definition) that the mutant in such cases has "4 per cent of dominance." This amount is so small that it has often been assumed that, for practical purposes, the mutant may be regarded as completely recessive. However, as will be seen in Sect. 20, even this small amount of dominance turns out to be very significant in deciding the way in which such mutant genes affect the population in which they occur. It must be understood, further, that the amount of dominance varies greatly from one type of gene to another, and that, for a given pair of alleles, it can in some cases be influenced to a considerable extent by differences in genes in other loci than their own, which modify the result.

As a result of the near-saturation potency of even one dose of most normal genes, hypermorphic mutations of normal genes would seldom produce an effect recognizably different from the normal. This fact, as well as the lesser probability of a change to greater than of one to lesser effectiveness (see p. 398) explains why such mutations of normal genes have so seldom been found. It also explains why genes are sometimes found which when homozygous give a normal phenotype, yet when crossed to a mutant hypomorph or amorph produce a heterozygote manifesting the mutant character to a distinctly greater degree than the usual normal does. These so-called *isoalleles* are hypomorphic enough, in com-

parison with the usual normal, so that in the heterozygote, or in single dose, the phenotypic level is distinctly below the saturation level, yet in the homozygote, i.e., in double dose, it appears to attain that level. In the case of certain genes (e.g., the normal allele of garnet eye in *Drosophila*) these cryptic mutants arise relatively frequently.

On the interpretation of dominance above given, it is to be expected that when two hypomorphic alleles of qualitatively the same type, lying at different points in the rapidly ascending portion of the phenotype-dosage curve, are crossed to each other or to an amorphic allele of the same gene, no marked dominance of one over the other would usually be shown. That is, the heterozygote or *compound* (as an individual heterozygous for two different mutant alleles of the same gene is called) would usually have a phenotype approximately intermediate between those of the two homozygous mutants. This has long been known to be the case, and is caused by the fact that both mutant genes in such a case are far from saturation potency, acting in the nearly straight-line portion of the curve relating gene dose or activity to amount of effect. Thus the addition of the two actions results in an intermediate amount of effect.

In some compounds, however, it has been found that the amount of effect cannot be explained as due simply to an addition of the actions of the two genes, working independently. As Stern and his co-workers (Stern, 1943; Stern and Heidenthal, 1944) have shown, the results in some compounds show an active interference or competition between the two alleles, indicating that there is in these cases a limited amount of substrate available, and that the higher ability of a given allele (or its products) to preempt this substrate, thus removing the latter from the possibility of being acted on by the other allele, is not always associated with a greater ability of the former allele to convert the substrate in a manner suitable for the production of the phenotypic effect that is under observation. Hence a more hypomorphic mutant allele sometimes has greater dominance than a less hypomorphic one. This has been found more especially in cases of changes in gene functioning caused by a position effect (*positional alleles*). Such results therefore occur oftener when mutants produced by ionizing radiation are used, since more of these than of spontaneous mutants result from structural changes rather than gene mutations. It is probable, however, that some gene mutations also have this kind of behavior. Effects of this kind could usually be detected readily only in compounds between two mutant alleles, since in heterozygotes having one normal allele the potency of the latter would usually be so high as to mask the effect of competition by the mutant allele. Some of these cases of interfering action appear to have a complicated basis, so that a series of compounds formed by a group of multiple alleles taken two at a time seems to show no consistent seriation of effects.

If the theory of dominance which has been presented is correct, neomorphic mutant genes, since they have not had an opportunity to be exposed to a prolonged natural selection for high potency, or for modifying genes in other loci that would tend to stabilize their effects, would usually find themselves in the near-linear region of the gene activity-effect curve. Hence marked phenotypic differences would usually result (1) when the dosage was changed, (2) when there were relatively slight changes in environmental conditions and/or (3) in genes of any one of numerous other loci than their own ("modifying genes"). Although the normal allele, not having an effect of the same kind, would not be dominant over them, neither would they show dominance over the normal but only the same sliding-scale result as when, in the absence of a normal allele, their dosage was changed. All this has been found to be the case. Thus the heterozygous neomorph is usually approximately intermediate in phenotype between the mutant and the normal homozygote, respectively. These findings then serve to confirm the general interpretation given in the foregoing discussion.

In *Drosophila*, where the matter has been most studied, the gene mutations produced by radiation have not so far appeared to show a different trend from the spontaneous ones in regard to any of these dosage or dominance effects. Moreover, the gene mutations originating from irradiation or spontaneously, when classified according to their types of dosage effects, have not shown obvious differences in their frequency distributions. Nevertheless it is necessary for those dealing with radiation mutations to be aware of these relations in order to know the way in which mutations induced in one generation become expressed in subsequent generations, and in order thereby to deduce (as shown in Sect. 20) the manner and speed with which they become either multiplied or eliminated from the population.

15. RADIATION AND SPONTANEOUS GENE-MUTATION FREQUENCIES IN *DROSOPHILA*

The determination of whether and to what extent radiation or any other agent or condition affects the incidence of mutations has depended upon the development of objective genetic techniques for the large-scale detection and counting of representative mutations and for distinguishing between newly arisen mutant genes or chromosomes and those previously present in a hidden heterozygous state in the stocks used. In *Drosophila* sex-linked lethal mutations, i.e., those in the sex-determining or X chromosome, were early found, in work of the present writer and Altenburg, to be especially suitable material for such a study (Muller and Altenburg, 1919, 1921; Muller, 1928c). The chief advantages of using them were that (1) the detection of sex-linked lethals already present in

a stock can readily be made, (2) the finding of lethals is subject to little subjective error, and (3) the frequency of origination of new lethals, even without special treatments, is high enough to be dealt with statistically. Moreover, evidence was found for regarding most of them as being no different in their manner of origin or type of genetic change from mutant genes in general, including those having a visible expression.

Since a male carries but one X chromosome, a sex-linked lethal in this chromosome results in his death prior to maturity, whereas in a female such a lethal in one of her two X chromosomes very seldom results in her death because it is strongly dominated over by the normal allele present in her other X chromosome. When she reproduces, half her sons will by Mendelian segregation receive from her an X chromosome with the lethal allele and they will therefore be killed, while the other half of the sons, receiving an X chromosome with the normal allele, will tend to live. All her daughters, however, will tend to survive, since even those getting the lethal will, like their mother, have in addition a dominant normal allele in the X chromosome derived from their father. Thus the existence of the lethal in one X chromosome of the mother can usually be recognized from the fact that she has approximately half as many sons as daughters. In *Drosophila* the criterion of a lethal is ordinarily made more definite than this by having that X chromosome of the mother which contains the lethal differ from her other X chromosome in regard to some gene or genes, termed *markers*, which have a conspicuous visible effect, and also by having crossing over between her two X chromosomes rendered ineffectual by the presence of an inversion in one of them. When such a female carrying a lethal is bred, all her sons inheriting the normal allele of the lethal, i.e., all her sons that survive, will exhibit the given marker characters that were in the X chromosome not having the lethal, whereas, if the female had carried no lethal, approximately half her sons would have these marker characters and the rest would have their alleles, giving the contrasting visible characters.

Essentially similar but more complicated techniques are employed for detecting lethals in chromosomes other than the sex chromosomes (the autosomes), but it is then necessary to find out whether or not individuals homozygous for the chromosome under investigation are able to survive. For this purpose a special scheme of inbreeding must be used, and the existence of the lethal, evidenced by the absence of a whole group of individuals of a given expected (marker) phenotype, can be ascertained only in the third generation of descendants. Moreover, the same kind of methods may, though with much more effort, be used for detecting genes which, instead of being fully lethal, are only detrimental. For, although individuals with the markers representing the chromosome in question are not completely absent in such cases, they may be significantly reduced in numbers, relatively to those of other types. Like-

wise, genetic schemes of this kind can be used for detecting steriles (mutations causing sterility) and visibles.

By the use of these methods it has been ascertained that in most cultures of *Drosophila* approximately one X chromosome in 600, on the average, contains a lethal gene that has arisen by "spontaneous" mutation during the immediately preceding generation. In contrast to this, if the heavy dose of 5000 r of ionizing radiation had been applied to the father's mature spermatozoa, approximately 14 per cent of the X chromosomes in them (a frequency 85 times as high as the preceding one) would have come to contain an induced lethal. According to the same data, if the frequency of induced lethals is proportional to dose (as will be shown to be the case in Chap. 8), it must take approximately 60 r, if applied to mature spermatozoa, to induce lethals at a frequency equal to that with which they usually arise spontaneously in the course of one generation, but the induced frequency would be added to the spontaneous one, thus doubling the rate of origination of the lethals.

These methods have also shown that not fully lethal but detrimental mutations having an effect marked enough to be detected by the methods used have a frequency of origination some three or four times that of the lethals, and that mutations in the autosomes arise (considered collectively) some four or five times as often as in the X chromosome. If these two facts are taken into account at once, the conclusion is reached that if all the mutations thus detectable are considered (visibles being here neglected as of insignificant relative numbers), their frequency is some 20 to 30 times that of sex-linked lethals. Hence their usual spontaneous frequency of origination per generation is in the neighborhood of one among twenty germ cells, and their frequency of induction by 5000 r applied to spermatozoa averages approximately four within each germ cell. By appropriate application of ultraviolet it is also possible to induce mutations at a frequency about as high as this.

These over-all frequencies of mutation can be translated into average frequencies per individual gene by dividing them by the total number of genes that participate in giving such mutations. However, the estimates of gene number thus far made may be inaccurate by a factor of about 2. Various methods indicate that in *Drosophila* there are some 5000 to 10,000 genes in a single (i.e., haploid) set of chromosomes. This would (assuming the great majority of genes to be capable of giving mutations of the already mentioned types) make the frequency of spontaneous mutation per individual gene per generation 1 in $20 \times 5{,}000$ or 10,000, that is, between 1 in 100,000 and 1 in 200,000; and that of mutation induced by application of 5000 r to spermatozoa 4×1 in 5000 or 10,000, that is, between 1 in 1250 and 1 in 2500.

A more direct method, called the *specific locus* method, of ascertaining the frequency of mutation per individual gene, is to take individuals

that are already supplied with "visible" alleles of certain chosen genes and cross them with individuals having the dominant alleles of the same genes, and then note how often offspring appear which show one of the given recessive characters. Such offspring ordinarily represent mutations of the dominant gene to one of its recessive alleles. These studies, although not yet carried out on an adequate scale for spontaneous mutations in *Drosophila*, nevertheless agree, as far as they go, with the estimate, arrived at by the above over-all method, of one spontaneous mutation per gene per generation being found among 100,000 to 200,000 germ cells. This is an average rate, since there are indications that the (detectable) rate is not the same for different loci. In contrast with this, the results obtained by the specific-locus method when radiation is applied are thus far discrepant by a factor of some 2 times with those of the over-all method. They have indicated that, on application of some 5000 r to spermatozoa, about one gene mutation is on the average (again with differences from locus to locus) induced per locus among some 3000 germ cells, rather than among the 1 in 1250 to 2500 indicated by the other method.

There are various possible reasons for this discordance. Among these are the considerable uncertainty regarding the number of genes, possible differences in induced mutation frequencies between the different stocks used, undoubted differences in mutation frequencies between different loci, and major technical difficulties in the detection both of detrimental and of visible mutations, as well as in the large-scale maintenance of stocks of them. Probably as important a difficulty as any of these arises from the fact that heavy irradiation of spermatozoa produces many minute deletions and other structural chromosomal changes. Many of these, in the case of lethals especially, are confused with the gene mutations. In the work with visible mutations at specific loci, on the other hand, far more of these cases were sifted out, both by breeding tests and cytological observations, and thus excluded from the count of mutations. This source of error is a much smaller one in the *Drosophila* studies on mutations produced by X or γ irradiation of ordinary interphase stages and in the studies on either ultraviolet or spontaneous mutations. In all these studies the great majority of the phenotypically recognizable types of genetic changes, including the lethals, fulfill present criteria for gene mutations. In such studies, however, the specific-locus technique has as yet been little used, because of the large-scale operations required for these applications of it.

A source of error of a different kind, which is present when the frequency of lethals arising in the X chromosome of ordinary interphase nuclei in premature (gonial or primordial) germ cells of the male are being studied, is that called *germinal selection*. This term refers to the fact that some of the lethals, especially those involving deficiencies, kill or retard

the proliferation of the very cells in which they occurred, through their effects on cell metabolism. Since the cells of the male contain but one X chromosome there is no normal allele present to dominate over these lethals. For this reason the mature spermatozoa that are finally produced, and that give rise to the offspring tested for mutations, sometimes come to contain only a third as high a frequency of mutant genes as has originally been produced. The amount of this germinal selection has been gauged by Kossikov (1936, 1937) and by Serebrovskaya and Shapiro (1935) by comparing the frequency of these mutations with the frequencies obtained in the autosomes of males or in either the X chromosomes or autosomes of females when the same early stages in germ cell history are irradiated. For the presence in these cases of a homologous chromosome, bearing the dominant normal allele, renders effects of the lethals on the metabolism of the cells containing them negligible.

Even when the error caused by germinal selection is avoided, by the use of one of the methods just referred to, it is found that lethals are induced by ionizing radiation in the gonial germ cells of *Drosophila* (representing for the most part ordinary interphase nuclei) with a distinctly lower frequency than in spermatozoa. A similar, although probably smaller, difference is found for specific visible gene mutations. In the case of the lethals, it is still unsafe to estimate what portion of the excess of the mutations in spermatozoa, as compared with earlier germ cells, is to be ascribed to the higher rate of production of deletions and other structural changes in them than in ordinary interphase nuclei. But, for the specific visible mutations, laborious analyses of the individual mutations have been carried out, in order to screen out the structural changes as far as possible. The preliminary results of this work (Muller, R. M. Valencia, and J. I. Valencia, 1950 and unpublished), which requires larger scale prosecution, indicate that the residual class designated as gene mutations is produced in ordinary interphase nuclei of germ cells (gonia) with only about a half of their ordinary frequency in spermatozoa. Oöcytes, at least, in their middle and later growth stages, and probably spermatocytes also, have an induced frequency of gene mutations more like that in spermatozoa than that in gonial stages (Berman, 1939; Muller, R. M. Valencia, and J. I. Valencia, 1950).[3] The results on visible gene mutations produced in the somatic cells of embryonic or young stages give frequencies of the same order of magnitude as obtained with germ cells, but they are as yet too meager for a decision as to which germ cell stage they resemble more closely in frequency. It is of course to be expected that they would have frequencies like those in the gonial (interphase) germ cells.

[3] The frequency in spermatozoa of different stages has recently been found to vary considerably, as noted in Chap. 8.

16. DIFFERENCES BETWEEN THE PRODUCTION OF MUTATIONS IN DIFFERENT SPECIES

As will be discussed in Chap. 8, some physicochemical conditions accompanying irradiation have a pronounced influence on the frequency of production by radiation of both gene mutations and structural changes. In view of this and of the foregoing results showing the influence of cellular stage, it is to be expected that some genetic differences also would influence these frequencies. Dubovsky (1935) reported finding statistically significant, although not very large, differences in the frequency of X-ray-induced lethals on irradiation of spermatozoa of different stocks of *Drosophila*. However, these results do not seem secure, when account is taken of various possible sources of error, such as the possible presence in some stocks of detrimental genes, which by a synergistic effect cause other detrimental genes, induced by the irradiation, to be classified as lethals. Similarly, much more striking differences in the induced mutation frequency observed in different stocks of bacteria may have had their origin in differences in the number of chromosome sets present ("ploidy") which affected the detection of the mutants. On the other hand Kossikov (1937) found sensibly the same X-ray-induced frequency of sex-linked lethals in *Drosophila simulans* as in *D. melanogaster* although here the genetic difference, being of species rank, must have been far greater than in any of the above cases. Timoféeff-Ressovsky (1931a) found only a slight difference in this respect between the much more widely separated species *D. melanogaster* and *D. funebris*. It therefore seems likely that large differences in induced frequency are comparatively rarely caused by such genetic differences as commonly exist between individuals of the same population.

There is much more reason for supposing marked differences to exist in the induced mutation frequency between organisms of widely differing systematic groups. The experimental establishment of this point meets with very serious difficulties, however. This is in part because comparisons of the over-all frequencies of any given phenotypic class of mutations are of very uncertain meaning. The differences in developmental and physiological processes between two organisms of very diverse kinds are so great that a given phenotypic category for one type of organism cannot be taken as being in genetic respects equivalent to any apparently corresponding category for the other type. Again, it has not as yet been possible, in organisms other than *Drosophila*, to obtain even an approximate estimate of the total number of genes, still less of the number of genes underlying any given "over-all" phenotypic category (such as lethals), and without such knowledge the significance of any comparative results on over-all mutation rates must remain unclear.

Nevertheless, comparisons of the work on widely different organisms

have provided some interesting points, both of agreement and of contrast. To note first some agreements, it is to be observed that lethals (as previously defined) appear among organisms in general to greatly outnumber the externally visible mutations, both in the case of spontaneous and of radiation-induced mutations. As for comparative frequencies in different organisms, the attempt to estimate total mutation frequency directly has been made only in *Drosophila*, for either spontaneous or induced mutations. However, certain spontaneous frequencies for specific visible loci have been determined for a number of organisms. From this work it has turned out, rather surprisingly, that the median frequencies per locus per generation for spontaneous gene mutations seem to be of the same order of magnitude in maize (Stadler, 1942), *Drosophila* (Muller, J. I. Valencia, and R. M. Valencia, 1950), mice (Russell, 1952 and unpublished), and human beings (Haldane, 1949). Nevertheless, in maize immense variations in spontaneous frequency between one locus and another appear to be very common, and the range covers three orders of magnitude, from the order of 1 in 1000 to 1 in 1,000,000 as found by Stadler, while in the three animal types mentioned the variation in frequency seems on the whole much smaller, although in certain cases still large, e.g., of one or occasionally even two orders of magnitude.

It is, however, when the mutations induced by ionizing radiation are examined that the most startling apparent differences in frequency are found. Most notable here is the fact that in the work on such mutations conducted by Stadler on maize since 1928, it has not yet been possible to obtain convincing evidence of the production of any gene mutations at all, either in the investigations of over-all mutation frequency or in those more critical experiments which deal with specific loci. It is true that so-called "point mutations," resembling gene mutations and inherited like them, were produced in abundance. Yet the results of analyses of mutations involving certain particular loci indicate that in the great majority of these cases, at least, the changes consist of minute deletions (Stadler, 1941; Stadler and Roman, 1948). This was evidenced by the facts that (1) the mutation induced at the given locus was always the most extreme, apparently amorphic, allele of the normal gene, unlike what was true of the spontaneous and ultraviolet mutations of the same gene, and (2) pollen containing the mutant genetic condition was always more or less deleteriously affected in their development or growth, like those with deficiencies (see p. 371) and unlike those with spontaneous or ultraviolet mutations of the given gene.

One important reason for this apparent dearth of induced gene mutations probably lies in the fact that, for a given dose of radiation, gross structural changes of chromosomes (including unrestituted single breaks) arise with far greater frequency, per cell, than in *Drosophila*. As these cause a high frequency of lethal effects, thus lowering the effective

fecundity, it is necessary to use a much lower dose than that commonly employed in *Drosophila*. Yet even at this low dose the frequency of minute deletions, like that of gross structural changes, is probably a good deal higher than for the same dose when applied to *Drosophila*. These deletions then would tend, by their numbers, to obscure from view the occurrence of gene mutations. Even when all these allowances are made, however, it still seems probable that gene mutations in maize, if produced at all by ionizing radiation, arise at a considerably lower frequency, for a given dose, than is the case in *Drosophila*. Nevertheless, in view of facts given in Chap. 8, there is theoretical ground for inferring that some gene mutations must be produced by this means even in maize.

17. AGENTS OTHER THAN RADIATION WHICH SEPARATELY AFFECT MUTATION FREQUENCY

In order that the mutagenic influence of radiation may be viewed in better perspective, consideration will be given in this section to the other agents which, acting without significant amounts of radiation, affect the occurrence of mutations. In this connection, it will be taken for granted that natural (earth and cosmic) radiation is ordinarily far too meager to play an appreciable role in the mutation process, and that therefore, in the absence of artificially applied radiation, these other influences are not working through any interaction with radiation effects. A review of the evidence for this will be postponed until the more detailed treatment of radiation mutagenesis presented in Chap. 8. For that chapter also will be reserved a discussion of the influence of other factors when they do work in conjunction with radiation.

The first condition of any kind found to affect mutation frequency was temperature (Muller and Altenburg, 1919; Muller, 1928c). *Drosophila* raised and bred at 27°C showed a frequency of origination of mutations (sex-linked and other lethals) per generation two to three times as high as did those at 17°C, despite the fact that in some of these experiments each generation passed at the lower temperature lasted two to three times as long. Thus, as worked out more exactly by Timoféef-Ressovsky (1935b), the temperature coefficient, Q_{10}, of the mutation frequency, was 5 to 6, a figure markedly higher than the Q_{10} of 2 to 3 which is characteristic of the processes of development and of metabolism, and of the reactions ordinarily dealt with by chemists.

This result could simply mean, as pointed out by Delbrück (1935), on entering this field from physics, that the chemical change involved in mutation has an especially high energy threshold, but takes place whenever this threshold is passed. If it is assumed that the frequency of mutation at a given temperature depends entirely on the frequency with which this threshold amount of kinetic energy is attained at that tem-

perature by the molecular and submolecular particles in the protoplasm in their collisions with the genes, and that any such superthreshold collision results in a mutation, then it can be calculated, as was done by Delbrück, that the mutation frequency of any given gene is so low—one mutation in some thousands of years, according to the present writer (1923)—as to indicate a correspondingly high energy threshold for the reaction. In fact, the threshold thus calculated from the mutation frequency turns out to be of just the right order of magnitude to result in the observed Q_{10} of 5 to 6. This correspondence with the observations lent support to Delbrück's interpretation.

Alternatively, it might be supposed that the mutation process requires contact between the gene and one or more special substances, perhaps also in a special way. In that case there would not necessarily be a particularly high energy threshold, for the given collisions might be very rare. As thermal agitation would in any case play a role in bringing about the required contacts, there would still be, other things being equal, an increase in mutation frequency with rise in temperature. But the increase caused in this way should be proportionate only to the speeding up of the life cycle and of general metabolism, thus following a Q_{10} of 2 to 3, unless the mutation process required the concatenation of more accidental events than did the other processes (i.e., unless it was essentially multimolecular).

In a case of this kind, however, "other things" might not be equal. That is, a difference in temperature might in addition influence the mutation frequency in another way, namely, by occasioning a difference in the concentration of substances which, directly or indirectly, affect the occurrence of mutation. Since substances favoring mutation would not necessarily become more concentrated with rise in temperature, it could not be predicted whether this influence, if it existed, would be in the direction of raising or of lowering the Q_{10}. It might, in fact, work in one direction in some types of organisms and contrariwise in others, and it might work differently according to just which temperatures were being studied.

In the above connection it should be noted that the lower and higher temperatures used (approximately 17° and 27°C) were both within the range that may be considered normal and innocuous to the organism. But, if this range had been transgressed in either direction, the circumstances affecting mutation frequency might well have been altered markedly. This is to be inferred from the considerations indicating that all organisms have been subjected to prolonged selection for a constitution which under normal conditions results in a lower mutation rate than would otherwise obtain in them. In consequence of this situation, marked departures from normal conditions, leading to disturbances in the biochemical organization, would on the whole tend to result in a higher

mutation rate. In apparent accord with this principle, later work has given evidence that under the influence of either abnormally high (Muller, 1928d; Plough and Ives, 1932; Buchmann and Timoféeff-Ressovsky, 1935, 1936) or abnormally low (Birkina, 1938; Kerkis, 1939) temperatures, and possibly also under that of violent changes in temperature even when the temperatures would, if constant, have lain in the normal range (Zuitin, 1939), the mutation frequency of *Drosophila* is considerably increased. Similarly, Stubbe and Döring (1938) have found malnutrition, caused by an undersupply of any one of several elements, to raise the mutation frequency in *Antirrhinum* two or more fold.

Meanwhile, long before the findings on abnormal temperatures, a second series of results on the so-called "spontaneous" mutation frequency in *Drosophila*, obtained by the present writer, had shown that it is a highly variable quantity, often differing from one experiment to another by a factor of 10 or more, and that at least one cause of this variability is probably the genetic composition (Muller, 1928c). Later, the existence of definite genes (doubtless themselves mutant) which increase the general mutation frequency of *Drosophila* to this extent was proved in several different investigations by Demerec (1937), Plough and Holthausen (1937), Neel (1942), and Ives and Andrews (1946). Such genes are now known as *mutator genes*. A gene of this kind in maize, called "sticky" from its effect on the chromosomes, in which it also caused a high frequency of breakage and structural change, has been reported by Beadle (1932). It is such mutations, affecting mutation frequency itself, but, in the main, those decreasing mutation frequency, which must have furnished most of the material for natural selection to work with, whereby the natural or "spontaneous" mutation frequency has been brought within such limits as are on the whole advantageous to the species in its survival and evolution. In addition, the more stable alleles of each individual gene must also have been selected.

The existence of marked variations in mutation frequency and, more specifically, of those caused by abnormal temperature influences, by malnutrition, and by mutator genes, pointed clearly to the conclusion that differences in chemical conditions must affect the occurrence of the mutations which are called spontaneous. It was therefore to be expected that mutagenic chemicals could be found, and that even the natural differences in cellular biochemistry associated with different stages of development, types of cell, and modes of metabolism might to some extent influence the occurrence of mutations. Nevertheless, tests of various substances, even in highly detrimental amounts, for a long time failed to give evidence of any marked production of mutations in *Drosophila*. It is true that results bordering on the significant, and indicating something on the order of a doubling of the mutation frequency, were occasionally reported (for example, in tests of iodine, of copper sulfate, and of ammo-

nia). In such cases, however, it was usually difficult to know, in view of the high variability of the spontaneous mutation frequency, whether differences in genetic constitution and in other conditions than the one under investigation had been rigorously enough ruled out. The same stricture applied to similar work which had been carried out by Stubbe, Baur and their co-workers on visible mutations in the snapdragon, *Antirrhinum* (Baur, 1924).

The first outstanding success in the use of a chemical for mutagenesis was obtained in 1941 by Auerbach and Robson in their tests of mustard gas and related substances on *Drosophila*, although the information could not be published in declassified form until 1946 (Auerbach, and Robson 1946 *et. seq.*). Robson had been led to suggest that tests be made to determine whether mustard resembles ionizing radiation in being mutagenic, from the consideration that its deep, slow-healing burns and other somatic effects showed, as he had noticed, a peculiar resemblance to the somatic effects of ionizing radiation. Moreover (as is now known) mustard, like radiation, also tends to inhibit mitosis. Like radiation again, it was found in Auerbach's tests to produce both gene mutations and structural changes of varied kinds, in considerable abundance. The former can in fact be induced by mustard with practically as high a frequency as by radiation. Structural changes, however, are induced somewhat more rarely than by doses of radiation giving the same frequency of gene mutations as the mustard. This may be because the chromosome breaks are not so nearly simultaneous in their occurrence as with radiation, being often delayed. In fact, unlike what happens with radiation, the mutation or chromosome change induced by mustard sometimes occurs in a chromosome that is a rather remote descendant of the one which had been directly treated, as though the treatment had originally induced in the genetic material a metastable state (like that of Baur's spontaneous "premutations" in *Antirrhinum*), in which the somatic effect was still normal, but which became copied in the process of chromosome reproduction, and finally resulted, in some of the descendant chromosomes, in a stable mutant configuration (Auerbach, 1947).

The mutagenic action of the mustard group is, like that of radiation, very general. It has now been proved for organisms of the most varied kinds, including bacteria, fungi, and (as far as structural changes are concerned) higher plants and mammals—in the latter two groups by Koller, Ansari, and Robson (1943) and Koller (1949). Since the discovery of mutagenesis by mustard, it has become difficult to avoid the conclusion that the similarity in the types of somatic effect of mustard and of ionizing radiation is, for most of the effects, based on the production of chromosome changes by both of these agents. On this view, it is to be expected that, like radiation, mustard would also have an especially

damaging action on more rapidly growing tissues. This has in fact proved to be the case. It now forms the basis for the use of mustard as an alternative or accessory to radiation in the treatment of malignancies. This is one way in which radiation genetics, this time through its offshoot, mustard mutagenesis, has had an important impact on therapy.

Closely following upon, and in part independently of, the work on mustards, an ever increasing series of chemicals has been found to have markedly mutagenic properties in given organisms, although few if any others are as potent, in doses which it is practicable to apply, as are the mustards themselves, and few others have yet been shown to be mutagenic in organisms in general. For some alleged mutagens, especially for some of the numerous substances reported to be mutagenic by Rapoport (1946a, b, 1948a, b) on the basis of independent work carried out by him in the U.S.S.R., confirmation is still lacking, but for other substances reported by him, as well as for a number of quite different substances, there is now no room for doubt, at least in certain organisms.

A notable, and probably the first, example found, following the mustards, of a substance that is probably mutagenic for organisms in general, is the narcotic ethyl urethane. It was discovered by Oehlkers (1943) that this substance produces chromosome changes in higher plant material, and it was discovered both by Rapoport (1946b) and by Vogt (1948, 1950) that it produces lethals, apparently of the gene-mutation kind, in *Drosophila*, and by Vogt that it also produces chromosome changes in *Drosophila*. In Rapoport's work, urethane was only one (although the most effective) of several carbamates found to produce mutations, and he appears to regard mutagenicity as characteristic of this entire group of substances.

In recent years the noteworthy fact has emerged that virtually all agents which have thus far been found to produce gene mutations, and for which the matter has been satisfactorily investigated, have also been found to have some effect in producing structural changes of chromosomes. This finding has recently been extended to a mutator gene in *Drosophila* by Hinton, Ives, and Evans (1952).

For a recent list of these chemicals the reader may be referred to Jensen, Kirk, Kolmark, and Westergaard (1952) and, for important additional substances, investigated by her, to Bird (1950, 1951, 1952). A bibliography of the extensive *Drosophila* literature on the genetic effects of chemicals has been published by Herskowitz (1951). An attempt to list chemical mutagens here would entail too great a digression, in view of the state of flux of this subject and the fact that, although many of them do fall into certain groups, these groups have not yet been found to show any agreement with one another in regard to features of either their structure or their mode of chemical or physiological action. Thus there is as yet no unified or agreed upon chemical interpretation of their mutagenesis,

nor has the course of chemical action been worked out in the case of even one mutagen, although there have of course been varied speculations. It is moreover likely that, in one sense, there can never be a unified interpretation, since it seems probable, a priori, that some chemicals would produce their biological effects through far more devious chemical pathways than others, yet happen to arrive at equivalent end results, perhaps through the same final pathway. An illustration of conditionality in mutagenesis is furnished by formaldehyde, since it has been found to cause a marked increase of the mutation rate when it is applied to the food of the *Drosophila* male, as shown by Rapoport (1946a) and confirmed by Kaplan (1948), yet it is ineffective when applied to the male at certain stages or under certain conditions, as shown by Auerbach (1949a) and by Herskowitz (1949), and quite nonmutagenic when applied to the female in any manner, as shown by Herskowitz (1950) and by Auerbach (1951).

Several whole groups of mutagens besides the mustards and carbamates do require specific mention here, however. Perhaps the first in importance is the group of organic and other peroxides, recently found to be mutagenic in both bacteria and molds, by Wyss, Clark, Haas, and Stone (1948), Dickey, Cleland, and Lotz (1949), and Wagner, Haddox, Fuerst, and Stone (1950). This group is of special interest for the present review because of the relation of radiation mutagenesis to oxidation. In fact, even oxygen itself has been found, by Conger and Fairchild (1952), to cause chromosome changes in *Tradescantia*. Possibly related in mode of action to the peroxides are certain other compounds, reported to be mutagenic in *Drosophila*. These include potassium permanganate, evidence for the mutagenicity of which was obtained as early as 1936 by Naumenko, and perhaps the epoxides, aldehydes, ketones, and even glycols, reported mutagenic by Rapoport (1946a, 1948a, b). However, the above-mentioned indirectness or special conditions of action found for one member of the aldehyde group—formaldehyde—when more detailed tests were made, shows that much caution is necessary in interpreting these results.

Recent tests of chemicals by Demerec, Bertani, and Flint (1951) have indicated that many different agents, when used in such concentration as to be very detrimental to bacteria (*E. coli*), occasion at the same time a moderate increase in their mutation frequency, by a factor, for instance, of 2 or 3. We are reminded here of the effects of both abnormal heat and abnormal cold, and of some reports of other detrimental conditions (see pp. 413–414), on *Drosophila* and on *Antirrhinum*. That is, the results indicate a biochemical disorganization in which the processes normally tending to hold the mutation frequency in check are to some extent interfered with. These agents, then, are not to be considered as being, like the mustards, directly mutagenic. The fact that this non-

specific effect in slightly increasing the mutation frequency has relatively seldom been demonstrated in *Drosophila*, even when such highly detrimental concentrations of chemicals are fed as to kill the great majority of them (Muller, 1928d), is probably due to the germ cells in multicellular animals being held protected in such a well-regulated somatic system that under many conditions death of the body as a whole occurs before the germ cells are allowed to have their metabolism greatly disturbed. Abnormal temperatures, unlike many chemicals, however, cannot be kept out of any part of such a small organism as a fly.[4]

18. INFLUENCE OF NORMAL METABOLIC PROCESSES ON THE OCCURRENCE OF GENE MUTATIONS

It would be of much interest to know the effects, if any, on the "spontaneous" gene mutation frequency which are exerted by the differences in metabolic processes and biochemical conditions generally which normally exist between different cells of the same organism, and between cells at different stages in the cell cycle, and in the life cycle of the whole individual.

In an approach to problems of this kind in *Drosophila* evidence was obtained by the present writer (1946a, b, but with the details still unpublished) that the occurrence of the great majority of spontaneous gene mutations in the germ cells, or cells of the germinal line, is concentrated into two relatively short periods of germ-track history. These are: (1) that of very early embryogeny, the so-called "early cleavage" stage when nuclear divisions are taking place in rapid succession; and (2) some period shortly antecedent to fertilization, either that in which the germ cells are undergoing their spurt of proliferation as gonia, just prior to their maturation, or the protracted maturation period itself, or both taken together. In the long intervening period of larval and adult life, in which by far the greater part of germ-cell existence is spent, very few mutations occur. This was shown by the fact that it made no perceptible difference in the final, total frequency of mutations whether or not this period is prolonged to several times its usual length, whether this prolongation takes place in the larval or adult stage, or whether the individual is starving or actively metabolizing and reproducing during that time. In addition to these two periods there is in the germ cells of the male one other period when spontaneous mutations occur at a per-

[4] Since the foregoing material was written, Novick and Szilard (1952) have reported distinct influences on the frequency of mutations in *E. coli* to be exerted by diverse substances, including some organic compounds of common occurrence in organisms, and others related to these. These important results, the obtaining of which was made possible by the use of their chemostat, have come to hand too late for consideration here.

ceptible rate, although probably not at as high a rate per unit of elapsed time as during the other two, namely, that of the mature spermatozoa. This is demonstrated by aging the spermatozoa, for when this is done it is found that the older spermatozoa carry more mutant genes.

The above striking correspondence between the periods of highest mutation rate and those of highest mitotic activity strongly suggests that either the process of gene reproduction itself, or at any rate some feature or features of the heightened metabolism associated with proliferation, are somehow conducive to the occurrence of mutations. Some evidence of this correspondence exists also in results from human material, if we may accept Haldane's (1947) calculation (based on certain Danish data on the incidence of a human mutation) indicating that the frequency of newly arisen mutant genes is much higher among human spermatozoa than among human eggs, inasmuch as there has been much more active proliferation in the production of the former than of the latter. On the other hand, the accumulation of mutations in aging spermatozoa is an example of a contrary kind, indicating that spontaneous mutations can also occur, at least under given conditions, when the genes are (as shown by Muller and Settles, 1927) in a state of dormancy.

One of the purposes of the experiment on aging in *Drosophila* was to attempt to throw light on the question, raised by the present writer (1928c), whether gene mutations consist in "mistakes" made in the synthesis of daughter genes by their mother genes, or in alterations of the already completed genes, or whether mutations of both types occur. The positive correlation between mutation frequency and mitotic frequency, both in *Drosophila* and apparently in man, might be interpreted as an indication of the mutations in that case being mainly of the first type, although it might alternatively be supposed that the greater metabolic activity of growing cells was in some way conducive to mutations in the completed genes. On the other hand, the apparent accumulation of mutations in mature spermatozoa would seem to indicate that in this case already formed genes had been changed. Yet it might, alternatively, be supposed in this case that the mutations occurred only after fertilization, in the process of construction of the daughter genes, under the influence of mutagenic substances accumulated during aging. It is true that on that supposition the mutant and normal genes would be mosaically distributed in the resulting individual, but the evidence as to whether or not this is the case is still lacking. Hence none of these experiments are conclusive so far as this problem is concerned.

More direct light on the question has been thrown by experiments with bacteria. In 1944 Zamenhof reported a correlation of mutation frequency in bacteria with frequency of reproduction. On the other hand, Novick and Szilard (1950), by means of highly refined methods involving the use of their "chemostat," succeeded in showing that, when reproduc-

tion of *E. coli* is hindered to various degrees by regulation of the supply of an essential nutrient, the frequency of mutations per unit of time remains constant. In other words, within a given length of time, as many mutants arise, on the average, in a line of bacteria that has undergone few divisions as in one that has undergone many, and this principle holds over a very wide range of proliferation rates. (A "line" in this case would signify a succession of individual bacteria produced one from the other by cell division, with just one of the two products of each cell division always being taken for the continuance of the line.) However (according to a personal communication from Szilard), when proliferation is reduced to zero, by the complete cutting off of the supply of the minimal constituent, mutations also cease, or nearly cease, to occur. The latter result may perhaps be used in reconciling the apparent contradiction between Zamenhof's findings and those of Novick and Szilard.

By analogy with the findings of Lederberg *et al.* (1952) on crossable strains of *E. coli*, in which genetic changes of an apparently similar kind could be shown by Mendelian analysis to involve individual genes, we may infer that the mutations dealt with by Novick and Szilard were in all probability gene mutations. Their finding of the independence between mutation frequency and reproduction rate within a wide range of the latter therefore implies that these mutations consisted of changes in already completed genes, rather than in the construction of new genes. Why then is it that the mutations occurred only when at least a little reproductive activity was going on? It is evident from the results that, as long as any proliferation at a rate above a certain minimal one is being attempted, but not in its absence, there is a steady stream of metabolic processes of some kind, not otherwise occurring, which result in occasional mutations. It is conceivable that these processes, in acting to cause a mutation, alter or rearrange gene material which, but for the mutation itself, would have remained in place just as it had been. But it would seem at least as likely that, in connection with these metabolic processes, a continual turnover and replacement of at least some of the gene parts is normally occurring, at a rate independent of the over-all growth, and that in the course of this replacement missteps occasionally occur, whereby a new gene part is substituted which is different, or which becomes arranged and connected up differently, from the old one that it replaces. The hypothetical normal replacement process might even involve a kind of gene reproduction itself. For if, as has sometimes been postulated, the gene produces its effects on the protoplasm by means of the building of partial or complete gene replicas, which become loosed into the cell, all the original gene material might not invariably stay behind in the chromosome while the newly built material emigrated, but a part or all of the old and of the new gene material might sometimes change places. In that case the mutations might after all consist in missteps in an actual

replication process. Light might be thrown on these questions by means of tracer studies. In the meantime, however, it must at least be admitted that these mutations do change the "old genes," in the more limited sense of not involving missteps in the formation of those daughter genes which are manufactured for the chromosomes of daughter cells.

Whatever interpretation of the above findings is preferred, there is one established series of results which only by a stretching of assumptions could be explained otherwise than by the occurrence of changes in already formed gene material. These are the data showing that treatment of mature *Drosophila* spermatozoa with ionizing radiation gives rise to mutant genes which are inherited by all parts of the offspring. If the mutations had been such as to involve only a change in the building of a daughter gene, then the offspring would be a mosaic, for it would have received a mutant daughter gene in one of the two nuclei of its "two-cell stage" and an unchanged mother gene in the other. In the case of some "visible" gene mutations, expressed in virtually all parts of the epidermis (and sometimes in some internal parts also) as a visible change in morphology or pigmentation, this mosaicism would be evident by causing a patchwork appearance of the mutant characteristic. Yet it has been found that, on the contrary, such gene mutations in the great majority of cases show their effects throughout all parts of the body which are capable of expressing them. The further fact that, in the spermatozoon itself, the gene lies in a dormant condition, makes it unlikely that the old gene becomes changed within the spermatozoon through a misstep in some otherwise normal process of turnover. It is true that a postponed turnover, occurring just after fertilization, might be invoked ad hoc, as an improbable alternative, to escape this conclusion. In that case, however, there would have to have been an intermediate step, in preparation for the mutation, and this step must itself have persisted unchanged throughout the spermatozoon stage; the subsequent misstep in turnover would then have to be very precisely timed, so as to take place before gene reproduction proper occurs in the fertilized egg. It is much simpler to suppose that the radiation permanently changed the old gene, within the spermatozoon itself.

19. RELATION BETWEEN MUTAGENICITY AND CARCINOGENICITY

As was indicated in Sect. 11, the question whether mutations (presumably "point mutations" of some kind) produced in somatic cells by radiation form the basis of the carcinogenic effect of radiation constitutes a part of the more general problem of whether somatic mutations, no matter how caused, result in malignancies. A further consideration of this topic has been deferred to this point because a number of the matters bearing upon it have been presented in our treatment, in the two preced-

ing sections, of conditions other than radiation which give rise to point (or gene) mutations.

If the view is held that some malignancies are caused by somatic mutations, it might be expected that some carcinogenic agents (all those which induced malignancies by this mechanism) would also prove to be mutagenic. With this in mind Auerbach and the present writer in 1939–1940 planned a series of experiments to test the mutagenic effect of methylcholanthrene and certain other carcinogenic hydrocarbons on *Drosophila*. In these experiments, carried out by Auerbach, the carcinogen was introduced parenterally. The genetic testing was done on a considerable scale. The results showed no perceptible increase in the frequency of mutations inherited by the offspring of the treated as compared with the control individuals. As was pointed out by Auerbach (1940), however, these negative results do not necessarily indicate a disjunction between carcinogenicity and mutagenicity, even in this case, since it is not known whether the given compounds or, indeed, any others can induce malignancies in *Drosophila*, or even whether "true" malignancies can occur in this organism, nor is it known whether the common carcinogens undergo chemical reactions in insects which are at all similar to those which they undergo in vertebrates.

This stricture still applies to all such work on the mutagenicity, in given nonvertebrates, of agents that are only known to be carcinogenic in vertebrates. In fact, this criticism may now be made stronger. The previously cited experiments with formaldehyde, for example, have given direct evidence that the mutagenicity of a given agent may be narrowly restricted to certain types of organisms; so too have experiments showing the mutagenicity of certain substances in bacteria and not in *Drosophila*. It is therefore not to be wondered at, or regarded as an objection to the somatic mutation interpretation of cancer, that, following Auerbach's first work on the subject, all other really critical tests of the mutagenicity of the carcinogenic hydrocarbons in nonvertebrate animals have given negative results. We disregard here certain apparently (though weakly) positive results, which were later shown to be nonreproducible (Demerec, Wallace, Witkin, and Bertani, 1949).

On the other hand, a much better test of the somatic mutation view would be the determination of whether carcinogens capable of inducing malignancies of varied kinds in vertebrates are mutagenic in the vertebrates themselves. Unfortunately, however, the tests of this sort on carcinogenic hydrocarbons which have so far been reported, although claiming to be positive, have not been carried out with sufficient rigor on the genetic side to give reliable results concerning mutation rate. On vertebrate material, the required work would have to be very large-scale, elaborate, and expensive, like, for example, that done on radiation

mutagenesis in mice by P. Hertwig (1939) or that by Russell, cited on pp. 411 and 432.

As for the study of carcinogenicity in nonvertebrates, it is still beset with too many doubts to provide critical data in this connection. In some of the work on *Drosophila*, for example, the criterion used for deciding that an agent is carcinogenic has been its action in causing an increase in the frequency of recognizable tumors of some special type in a mutant strain already having, even without treatment, a high incidence of tumors of this particular type (Burdette, 1951). It is uncertain, in the first place, whether these tumors can be considered malignant, in the sense in which this term is used when applied to vertebrates. Secondly, the observed differences in the frequency of detected tumors may have been caused by influences affecting the amount of their growth and melanization rather than that of their origination. Finally, even if the agent did affect the frequency of origination of the tumors, it might be able to exert this influence only through some interaction, perhaps confined to that particular type of tissue in which the given tumor arises, between it and the products of the very special genetic agent possessed by the mutant strain used. Hence the results of these tests, no matter whether showing a parallelism or a lack of parallelism between mutagenicity and this putative "carcinogenicity" in *Drosophila*, must be regarded as far from definitive in their bearing on the somatic mutation interpretation of cancer.

There is, fortunately, a type of test available of the relation between mutagenicity and carcinogenicity which is much less subject to difficulties and objections of the various kinds mentioned. This consists in the determination of whether agents which have given evidence of producing point mutations in *organisms in general* act also as carcinogens, in those forms in which the de novo origin of indubitable malignancies can be definitely recognized. It was the discovery of the mutagenic effect of ionizing radiation, an agent already known to be carcinogenic, which had provided the first factual evidence in support of the somatic mutation hypothesis of malignancies—a relation first pointed out by the present writer (1927)—and the results of other workers, showing that radiation is mutagenic in organisms in general, served to make this evidence much more definite. The evidence then received a further important extension in the results showing that ultraviolet radiation also is a general mutagen, inasmuch as this agent likewise was known to be carcinogenic in vertebrates. The lesser yet positive effect of high temperature, in promoting the origination of both mutations and cancers, pointed in the same direction. But it is obviously a requirement of the somatic mutation view of malignancies (even though it be admitted, as it must be, that only some malignancies are of such origin) that *all* agents which produce point

mutations in organisms in general should in addition prove to be carcinogenic in vertebrates. Thus the recent finding of chemical mutagens has furnished an unexampled opportunity for the further testing out of the hypothesis along these lines.

For a long time it had been thought that chemicals of the mustard group were not carcinogenic. However, more adequate tests, recently carried out at the Chester Beatty Research Institute in England (Bird, 1949, 1950; Boyland and Horning, 1949), at the U. S. Institutes of Health (Heston, 1949, 1950) and at Stanford University (Griffen, Brandt, and Tatum, 1950) have now left no doubt of the efficacy of nitrogen mustards and of other mutagenic mustards in inducing malignancies of varied kinds. Moreover, as for urethane, it has long been known that this substance is carcinogenic. This striking series of correspondences can hardly be dismissed as mere coincidence, and therefore serves greatly to strengthen, if not actually to clinch, the argument for the mutational interpretation.[5] Fortunately, however, for the removal or confirmation of residual doubts, the way is still open for the ready extension of this line of investigation by the testing of the carcinogenicity of other substances for which evidence of a generalized mutagenicity is obtained. Moreover, it still remains to be determined whether conditions which, when acting along with mutagens, under all circumstances increase or decrease the efficacy of the latter in mutagenesis, have a corresponding influence on their carcinogenic potency also.

On returning to the original question of whether the effect of radiation in producing gene mutations forms the basis of its effect in producing malignancies, it will be seen that a comparative survey of the results not only with radiation of different types but also with other agents now makes this view—better, in the light of these results, to be termed a theory—highly probable.

20. MANNER OF ACCUMULATION, EXPRESSION, AND ELIMINATION OF MUTATIONS

Gross structural changes of chromosomes involving translocations or gross inversions usually result (as pointed out in Sects. 5–6) in a

[5] At the same time, the above conclusion in no way casts doubt upon the fact, long since demonstrated, that some malignancies are caused not by gene mutation but by the influence of parasitic microorganisms or viruses, some of which may under other circumstances or when possessed of a somewhat different genetic composition be non-malignant or even symbiotic. It is in addition possible, although at present in the realm of speculation, that in some cases changes in plasmagenes, or in autocatalytically replenished substances originating in the organism itself [like the antigens studied by Sonneborn (1948, 1950) in *Paramecium*], form the basis of malignancies. The chromosomal genes of the organism itself, however, by virtue of their number, variety, and stability in transmission through cell division, afford the greatest amount of diversity, range of effect, and material for the accomplishment of such changes.

certain proportion of the offspring of individuals heterozygous for them receiving aneuploid chromosome combinations which kill off these offspring in embryonic stages, and this lowered productivity reduces, from generation to generation, the relative number of individuals carrying such aberrations, until those aberrations which arose in any given generation have finally become eliminated from the population. Although in primates and especially in civilized man this elimination is, as previously explained, much slower than in most organisms, it is even in them probably more rapid, especially for translocations, which constitute the great majority of these aberrations, than the rate of elimination (discussed in the following pages) of most mutant genes. At the same time, in mammals the heterozygous carriers of the translocations and inversions probably do not suffer from any somatic ill effects caused by their aberrations, since in mammals, as in most organisms studied, chromosome changes are not likely to be associated with position effects at all, much less with dominant position effects.

Elimination of the above type, occurring only through death of embryos, constitutes less of a burden on any population than when, as in the case of most mutant genes, the elimination occurs in the later stages of the abnormal individuals. For the older abnormals prior to their elimination engage in a competition with the normals which is more detrimental to the latter. Moreover, from a human viewpoint, the elimination of embryos is also less objectionable than that of older individuals because of the fact that in such cases the abnormals die before they themselves have had a chance to suffer consciously from the effects of their abnormality.

Gene mutations are not only individually more damaging and objectionable than gross chromosome aberrations, for the reasons given, but they are also far more frequent and more diverse in their phenotypic expressions, both when they occur spontaneously and when they are produced by irradiation—except in the special case of irradiation of mature spermatozoa, when chromosome aberrations arise with a frequency comparable to theirs. It is therefore appropriate to consider in some detail the manner in which the resulting mutant genes affect the individuals inheriting them and the population in general throughout the course of any number of generations.

There are usually many circumstances, both nongenetic and genetic, besides the possession of some given mutant gene which decide how many offspring an individual produces, and each offspring has only a 50 per cent chance of receiving from its parent any mutant gene for which the latter is heterozygous. Since this remains true generation after generation, a given mutant may in the course of time become more or less multiplied in the population or may become eliminated from it, quite apart from any detriment or benefit conferred by the gene. However, those

respective chances of multiplication and elimination which are independent of the effects of the gene in question are equal in amount, and so they tend to compensate each other exactly in the long run, when the results for many mutant genes, present in a large population of stable size, are added together. Thus, for a large group of hypothetical mutant genes which conferred neither detriment nor benefit on their possessors, their collective frequency in the population after any given number of generations would still be approximately equal (subject to some statistical deviation) to their frequency in the first generation considered (e.g., in the generation in which they originated), even though some of the genes had become multiplied and others, in compensating number, had become eliminated. That is, each individual mutant gene present in the beginning generation would *on the average* be represented by just one descendant gene in the nth generation.

However, for the vast majority of mutant genes, those which confer some disadvantage, even though slight, and also for the very rare ones which confer an advantage, the situation is modified by their somatic effect. Considering a large group of mutant genes, all of which give rise to one or another impairment of such magnitude as to reduce the average chance of reproduction of an individual containing such a gene by a given amount, i, which we may, for example, imagine to be 10 per cent, it is evident that after one generation of breeding these genes will have a frequency of approximately $1 - i$ in the population, after two generations one of $(1 - i)^2$, and after n generations one of $(1 - i)^n$. By summation of these frequencies over an unlimited number of generations it is readily shown that each such gene is transmitted, on the average, to a total of $1/i$ individuals. Thus, for genes whose $i = 10$ per cent, giving 1 chance in 10 of elimination in each individual, the total number of individuals which in the course of successive generations come to inherit a descendant of this mutant gene before it is eliminated from the population is on the average 1/0.1, or 10. The value $1/i$ is designated as the *persistence*, p, i.e., $1/i = p$, and in our example $p = 10$.

These considerations show that each mutant gene that exerts a disadvantageous over-all effect, no matter how small, is eventually eliminated, i.e., it leads to a "genetic death" by prematurely killing off or preventing the reproduction of, on the average, one descendant containing it.[6] Moreover, the number of descendants that contain and are hampered by a given mutant gene is on the average exactly the reciprocal

[6] The death is to be considered as only a "half death" and the total load as only a half of one unit of load for genes which are so recessive that their elimination occurs in individuals homozygous for them, since in this case the cooperation of the gene from the other parent is required for the effect. As pointed out in the text, however, it is probable that the great majority of mutant genes, even when seemingly of a recessive type, have enough dominance to be eliminated in heterozygous individuals, and therefore give rise to one death, and one unit of load.

of the amount of detriment it produces, as measured by the average risk of genetic death which it gives rise to in individuals carrying it. For this reason the total "load" or disability imparted to the population, in the course of a succession of generations, is in the end as much for a mutant gene which has a slightly detrimental effect as for one which has a highly detrimental or fully lethal action on the individuals possessing it. The average amount of this total load may be said to add up to unity, i.e., the complete disability of one individual, for each detrimental mutant gene which is received by one offspring of the first generation after its origination.[6] This unit total load is in the case of slightly detrimental genes distributed in smaller fractions per individual but over correspondingly more individuals, than it is in the case of more markedly detrimental genes.

In the above treatment no consideration was given to whether the mutant gene was dominant or recessive; hence the value for impairment, i, as used, had to be taken as an average for the effect in all individuals having the gene, including both those heterozygous and those homozygous for it. The value was therefore in part dependent on the numbers of these types relative to each other. Actually, however, it is usually the amount of impairment in the heterozygote which plays the decisive role. It can be shown that most mutant genes very seldom become homozygous, since they exert enough detrimental effect (even though this is very slight) when heterozygous to become eliminated before they have a chance to exist in homozygous condition. In this sense they are said to be *effectively dominant* (Muller, 1950b).

In *Drosophila*, genes that are lethal or nearly lethal in homozygotes commonly produce an impairment of some 2 to 7 per cent, or roughly $\frac{1}{25}$, in heterozygotes (Stern *et al.*, 1948, 1951, 1952; Muller, 1950b, c). Thus for the lethals in heterozygotes $p = 25$; i.e., each such gene tends to pass down through some 25 individuals, on the average, before being eliminated. As the frequency, among the germ cells of the general population, of already existing lethals occupying any given locus is usually far less than 1 in 25 (as will be shown in what follows), the chance is small that before its elimination any given lethal will meet another of the same kind at fertilization and thereby become homozygous. Much the greater part of the damage done by such genes in the population is therefore made up of the collective impairment exerted by them in heterozygotes. There is reason to infer that those mutant genes, much more numerous than lethals, which are less detrimental than lethals to individuals homozygous for them, have in general relatively more expression in heterozygotes, as compared with that in homozygotes, i.e., that they have a greater tendency toward dominance than the lethals. Moreover, this tendency is probably more marked in man than in *Dro-*

[6] See opposite page for footnote.

sophila. Hence for detrimental mutant genes also it usually turns out that their effect in heterozygotes is the important factor in determining the damage they do in individuals, and the amount of their persistence in the population. For these reasons we may usually, for practical purposes, simplify our calculation by using for the value of i the amount of impairment in heterozygotes alone, and by taking p as the reciprocal of this value of i.

Individual mutant genes that have been produced by irradiation of individuals of a given generation are very seldom to be detected by means of recognizable effects in the offspring, or indeed in any later generation descendants, in any mixed population like a human one, which undergoes relatively little inbreeding. This is because of a combination of reasons. First is the rarity with which the mutant genes become homozygous and thereby more marked in their effects; in fact, those whose effects when homozygous would be especially marked and recognizable are the very ones which tend to be eliminated sooner as heterozygotes and therefore to occur least often as homozygotes. Second is the fact that, even as homozygotes, most mutant genes have effects that are not readily detected. Third, their effects in the heterozygous state, that in which they usually exist, are much weaker and less recognizable still, commonly involving, as far as the ordinary observer is concerned, only a small quantitative difference from the phenotype that would otherwise be present. Fourth, these slight effects are superimposed upon the innumerable variations which would be present anyhow in any such population. These other variations are caused both by the segregation and recombination of the many mutant genes accumulated from scores of past generations during which spontaneous mutations have been occurring, and also by the operation of environmental factors, such as disease, nutritional differences, mode of life, etc. For these reasons it is doubtful whether it would be practicable, even by the large-scale study of such populations, to demonstrate that mutations had been induced in them, even if these mutations had been relatively abundant and, in their collective effect over the course of many generations, highly damaging.

It is sometimes assumed that a "unit" genetic load which is distributed in very small fractions among very many individuals may be regarded as of negligible consequence, in comparison, for example, with a single case of major affliction leading to premature death. However, this idea is an erroneous one. It is true that the amount of actual damage done to life or reproduction in cases of genetic impairment is often subject to a high statistical variation, so that in cases of slight impairment the damage is for some individuals nil, but such cases are compensated for by others in which the individual incurs more damage than that calculated, so that the average amount of damage is maintained. The reality of the small effects may be better realized when the fact is considered that prac-

tically all individuals in any population are having their ability to live and reproduce reduced considerably below that of hypothetical individuals homozygous for normal genes exclusively, and that the greater part of this reduction is usually caused by the collective action of multiple, individually minute, fractional "loads," most of them occasioned by heterozygous mutant genes. Yet even the relatively small part of the total load on an individual which is caused by homozygous genes (again usually minute and unrecognizable in their individual action, even though homozygous) is often quite sizeable. This is shown by the marked increase in size, vigor, fertility, and general viability (including even, as shown in unpublished work of Russell's on mice, resistance to the lethal effects of radiation), which often results from crossing widely different strains—a phenomenon known as *heterosis* and utilized extensively in the production of hybrid corn, poultry, swine, etc. If, then, the mutant genes could have been removed even in their heterozygous state and replaced by normal ones, the results would have been far more remarkable, in view of the fact that much the greater part of the load is usually in this form. Since now these very large differences are mainly due to the cumulative action of numerous mutant gene effects, each of which is so tiny that it cannot be detected individually, it follows that the individual effects are real, significant, and cumulative.

It is of interest to estimate the total collective magnitude of these effects per individual in an ordinary population, and the risk of genetic extinction therewith entailed, and then to compare these figures with those, to be superposed upon them, which represent the genetic effects produced by a given amount of radiation. The theoretical calculation is basically a simple one. It has been noted in the foregoing that each mutant gene which passes into the population, no matter how slight its detrimental effect on an individual possessing it, is finally eliminated by reason of that detrimental effect, usually in a heterozygous individual. In consequence of this, there must in the long run be about as many genetic deaths per generation in a population as there are mutations arising in it, i.e., 2μ (if μ represents the mutation frequency in the germ cells), minus the frequency of excess mutant genes contained in cases of what may be termed "overlapping" genetic deaths, explained in the next paragraph. The factor 2 here arises from the fact that a heterozygous individual (homozygotes being here considered to be of negligible frequency) can be affected by a mutant gene received from either one of the two germ cells from which that individual originated. Moreover, looking at the matter conversely, the genetic death of the heterozygote, since he comprises two genomes (sets of genes), one of which has the normal allele, reduces the per-genome and therefore the per-germ-cell frequency of the mutant gene by only half as much as it reduces the frequency of individuals bearing the mutant gene; hence, in order to effect sufficient gene elimination

through heterozygotes to compensate for the mutations arising, twice as high a frequency of them must be eliminated as the frequency μ that would be necessary if the elimination could be carried out in the germ cell stage.

The term "overlapping" as applied to genetic deaths refers to two types of cases. In the first, which may be called the cases of "independent overlapping," an individual who meets genetic extinction through the effect of a given gene would have met extinction anyway through the effect of one or more other independently acting genes which he also happened to carry, so that the one death accomplished more than one effective gene-elimination. In the second, which may be called the cases of "dependent overlapping," the individual's genetic survival is prevented by a synergistic (i.e., more than factorially cumulative) action of two or more mutant genes, which thereby exercise a kind of economy in causing extinctions. The frequency of independent overlapping can readily be calculated from the mutation frequency and turns out to be relatively unimportant unless μ exceeds 0.1. Where, however, (as might be the case in man) μ is as high as 0.5, independent overlapping would reduce the frequency of genetic deaths of individuals from 1.0 ($= 2\mu$) to about 0.63. Dependent overlapping seems hitherto to have been ignored in calculations of elimination rate. Although probably important, its amount of influence cannot at present, in the absence of empirical data on the subject, be estimated. It seems very unlikely, however, from calculations based on plausible assumptions as to the frequencies of genes having various grades and multiplicities of synergistic interaction, that it would reduce the elimination rate of individuals by a factor of more than 2 or 3, and there is more latitude than this anyway in present estimates of the human spontaneous mutation rate. For the present, then, the extent of this influence will have to be left doubtful.[7]

There are reasons to conclude (Muller, 1950b) that 0.1 (i.e., 1 germ cell in 10) represents a low minimum for the per-generation frequency of new spontaneous mutations among human germ cells; 0.2 or even 0.4 appear much more probable figures, and some competent students of the subject believe the rate to be considerably higher yet. Taking 0.3 as a probable value not on the excessive side, 2μ becomes 0.6, and, if the seemingly high allowance of a factor of 3 is made for overlapping, of both the types just discussed, we obtain 0.2, or an average of one person in every five, as the frequency, probably minimal, of individuals being genetically eliminated. This would apply in a human population that was maintaining a constant frequency of mutant genes.

The condition of constant or *equilibrium* frequency of mutant genes can

[7] The possible importance of what we have termed "dependent overlapping" was recently pointed out by Neel and Falls (1951), and also in a personal communication received from Altenburg.

of course be violated for a time, but it cannot be violated indefinitely, in the direction of increase, without finally causing extinction of the population. When, for example, individuals who would otherwise have been eliminated are saved for reproduction by medical and other artificial aids, the elimination rate temporarily falls below that of origination of new mutant genes, so that the frequency of mutant genes in the population is gradually raised. This increase continues until individuals become heavily enough afflicted to undergo genetic elimination, despite the artificial aids, at a rate which is again equivalent to the old rate of 2μ, modified by the overlapping, at which they are still receiving new mutant genes. Hence 0.2 or some related value, most likely higher, remains the figure representing the necessary long-term genetic elimination rate in man as long as the mutation rate remains what it now is.

The figure for elimination rate represents also the average amount of genetic disability suffered in the long run by the individuals of the population. That is, utilizing the above figure, the individuals must on the average have an amount of disability commensurate with this one chance in five of genetic extinction. The figure 0.2 does not mean that one individual in five carries some one gene which regularly causes death or failure to reproduce. For it was shown above that the great majority of the genes which arise by mutation give, individually, only a small chance of extinction but have a correspondingly high persistence, thereby becoming distributed among many individuals. Thus each individual comes to have many of these genes, enough to make the average individual's chance of extinction, caused by all his mutant genes collectively, one in five. The average frequency of the mutant genes per individual of the population must (ignoring the occurrence of homozygotes) be 2μ times the average persistence p of these genes. Although we are far from knowing the value of p, it is almost certainly several score and more likely at least 100. That is, each mutant gene is probably distributed to at least 100 individuals, on the average, before it dies out. With μ taken as 0.3 this would cause each individual to carry, on the average, at least 60 deleterious heterozygous genes, usually of individually small effect.[8]

In view of the fact that the distribution of the mutant genes with respect to each other is on the whole a random one, the number of genes in different individuals would tend to follow a Poisson (random) distribution. Thus, with an average value of 60 per individual, the numbers in different individuals would not range very widely about this, having a standard

[8] It is true that the comparatively low figure of 8 was presented by the writer in an earlier paper (Muller, 1950b), but, as was there pointed out, this represented an attempt to find a "rock-bottom" minimum value, based on assumptions that were almost certainly too cautious. The above figure of 60, on the other hand, results from an attempt to find a value based on assumptions regarding mutation rate and gene action which seem more probable.

deviation of not quite ± 8. It thus becomes evident that the individuals, although varying from one another somewhat, tend to suffer from comparable total loads, there being, in most cases, no very large distinction between the person whose disability happens to lead to elimination and the one who escapes it. The genetic extinction, then, is seldom caused by some unusual all-or-none condition or process, but rather by a kind of generalized inadequacy, shared to a considerable degree even by the individuals who escape extinction. There must, to be sure, be certain features—forming a pattern of defect—which are more marked in one case, and others in another, but only in relatively rare cases will a single feature or syndrome greatly predominate in providing the risk of extinction. This situation shows that the figure for elimination rate, no matter whether its true value is more or less than 0.2, does not usually represent some major peril which strikes in occasional spots, but rather, for most individuals of the population, including those who survive genetically, a more or less generalized, continuing handicap, inasmuch as it is compounded of so many small moieties acting in different yet cumulative ways. It is in this sense legitimate to regard this figure as representing a a true "average disability," borne in large measure by the great majority of individuals. It is a handicap of the given amount, however, only by comparison with the potential performance of hypothetical individuals free of mutant genes.

21. MANNER OF INCIDENCE OF RADIATION DAMAGE IN SUBSEQUENT GENERATIONS

How is the above picture altered when additional mutations are produced by exposure to radiation? Suppose that an entire population had been exposed for one generation to a dose which in the gonads averaged 20 r, and that, as Russell's preliminary data on mice indicate, this caused mutations at a frequency of about one-quarter of that occurring spontaneously in man. Taking the spontaneous μ as 0.3 would make an induced frequency of 0.075. If the 0.3 spontaneous mutations of each generation give rise to an elimination of individuals represented by the figure 0.2, as explained above, the additional 0.075 induced mutations would give rise to an approximately proportionate elimination of about 0.05. That is, if the given population comprised 100,000 individuals per generation, a total of about 5000 would eventually suffer genetic death by reason of the mutations induced in that one generation, in addition to 20,000 who would suffer genetic death anyway by reason of the spontaneous mutations that had occurred in the same generation. These 5000 induced eliminations, however, like the 20,000 "natural" ones due to the spontaneous mutations of that particular generation, would be scattered out over scores and even hundreds of generations of descend-

ants, with perhaps not more than 50 of the 5000 induced genetic deaths occurring in any single generation. Since the natural genetically caused elimination rate of the population in the equilibrium state is 20,000 per generation (a collective figure involving the elimination of some genes derived from mutations in each of scores or hundreds of past generations), the addition to it of this 50 or fewer genetic deaths would probably be imperceptible. So too would the added disabilities, which would, on the average, increase very slightly (by but a few per cent) the "load" carried by $5000 \times p$ or (substituting 100 for p) 500,000 persons, scattered over many centuries. That is, the damage would be entirely real, and of great over-all magnitude, yet not to be detected because so exceedingly dispersed.

If, however, the 20 r exposure was continued generation after generation, the effect of the added mutations would eventually become important for each generation. A new equilibrium rate of elimination would finally be reached, some 25 per cent higher than the original one, to match the constantly higher mutation rate. At the new rate 25,000 persons would be genetically eliminated in *each* generation instead of 20,000, a quite noticeable increase. At the same time the average amount of disability per person would also have grown so that, if before it could be represented by the figure 20 per cent, it would now have risen to 25 per cent.

It is not known how much permanent increase in mutation rate any given species is capable of enduring without decreasing in numbers and finally dying out, but each species has its own limit, determined by the magnitude of its original mutation rate and the rapidity with which it can replace lost numbers by selective multiplication. There are grounds for inferring that man, by reason of the high spontaneous mutation rate that he already has, coupled with his slow natural rate of multiplication, particularly under conditions of modern civilization where the birth rate is artificially reduced and genetic deaths interfered with, may already be near if not beyond that limit, which may be called the *critical mutation rate*. Much research is required before the facts relevant to a decision of this question can be determined with sufficient exactness. If it is true that the limit has been closely approached, then even such an increase of mutation rate as the 25 per cent above postulated might transgress it, and could not be tolerated indefinitely.

It might be thought that since the genetic damage of radiation becomes so widely dispersed, without greatly affecting the immediate descendants of an exposed individual, consideration should be given only to the total amount of exposure of the population as a whole, and perhaps only to the total incurred over the course of many generations, so as to keep this total within limits that are not dangerous to mankind as a whole. This limiting "permissible total dose," whatever it is taken to be, when divided by the total estimated population of all the parental generations in ques-

tion taken together, would become converted into a "permissible average dose." Individuals might then be allowed, as far as genetic considerations were concerned, to receive more than this permissible average, provided so many others received less that the average amount received by all did not rise above that held to be permissible. It is certainly of major importance, from the viewpoint of humanity in general, to draw some such over-all line. It should be based on such considerations as the critical mutation rate or the amount of genetic load to be regarded as tolerable. On the other hand, every increment in the genetic load must be regarded as in itself objectionable, and to be avoided if possible, even if it does not threaten to wipe out mankind as a whole, and even if it is distributed in such a way that it cannot be recognized as such.

It is in addition desirable to realize how much damage to descendants the exposure of any one given individual may give rise to, in order that it may be decided whether the benefit or chance of benefit to be derived from one or more proposed exposures of this individual is enough greater than that to be derived from alternative procedures to justify taking the risk of damage in the given case. In order to assess this probable damage, it is necessary to have an estimate of the chance of the production of mutations, and of their inheritance by one or more offspring. To set against this the probable benefit also should be assessed.

An example will be used to illustrate the theory to be followed in such a case. Let us assume, as was done in the foregoing, that a dose of 80 r received by the immature germ cells of human gonads gives a 0.3, or 1 in $3\frac{1}{3}$, risk of inducing a detrimental mutation in any of these germ cells. Suppose now that the question has been raised whether a given young woman who has hitherto been unable to ovulate successfully should have her ovaries treated with a dose of some 275 r of X rays, as is sometimes done in such cases, in the hope of enabling her to fulfill her strong desire to have children. It will be seen that this dose, being approximately $3\frac{1}{3}$ times 80 r, will produce an average of about 1 detrimental or lethal mutation per germ cell. If the woman thereafter succeeds in producing 3 children these will (in the average of such cases) carry three induced mutations (one each, most probably), in addition to those mutant genes which would have been in them anyway. There is a negligible chance that these children, or their children in turn, would be perceptibly affected in consequence of these induced mutations. However, three "unit loads" would have been created which would, on the average, be passed down to three lines of descendants, until finally three genetic deaths occurred or if, to be conservative, the maximum plausible allowance is made for the overlapping of gene effects previously discussed, one case of genetic death. Moreover, there would in the meantime have been a long series of small disabilities (added to those which would have been present anyhow), which were collectively equivalent, assuming this much overlapping, to one total disability. Thus the net result of a series

of such cases would be that the frustration of the present potential mothers would have been exchanged at the expense of an equal or greater total amount of human frustration in later generations.

From these calculations it will be seen that the procedure, followed by some physicians, of exposing the testes of relatively young men to a dose of some 500 r, in order temporarily to check the reproduction of those who intend to beget children at a later period, would probably, according to the best but admittedly not yet good enough present indications, result in the equivalent of several frustrated future lives, on the average, for each person enjoying this convenience. It should further be observed that the eventual individual victims in all these cases would not have been spared by the exercise of more caution in the exposure of the rest of the population, with the intention of ensuring that the average dose received by the population as a whole did not rise above the agreed upon limit of 20 r, or whatever it may have been taken to be. Moreover, if less radiation were really used in other cases, to compensate for the large doses necessary for these partial sterilizations, many people might be penalized by being deprived of the benefits of radiation which could have been put to better use in their cases.

When the dose received is less than enough to produce one mutation per germ cell, it can nevertheless be expressed as a risk, or as resulting in an average of one mutation in a certain number of offspring, in a series of cases of the given kind. This damage or risk of damage (Muller, 1951a) is then again to be compared with the average benefit, or rather, with the amount by which the average benefit would exceed that to be derived from the best alternative procedure. In many cases the alternative procedure may itself involve irradiation, but irradiation applied with special precautions, such as (where it is intended primarily to expose somatic parts) shielding of the gonads, shuttering down of the field, limitation of the time and amperage of exposure, and adjustment of the voltage so as to give the least exposure to other parts that is commensurate with sufficient exposure of the parts in question. In such cases a considerable decrease in genetic risk may sometimes be achieved while the only decrease in benefit to be set against it will be the inconvenience attached to the taking of the special precautions. Even for very small exposures such precautions are usually justified, for these are the very ones which are most likely to be relatively often repeated, and it is the total accumulated exposure, received over an unlimited period prior to reproduction, which counts in determining the chance of producing a gene mutation which will be inherited (as explained in Chap. 8).

22. SPEEDING UP OF EVOLUTION BY IRRADIATION

In all the above discussions only the harmful mutations have been taken into consideration. Even though beneficial mutations form but an

exceedingly minute fraction of the total, they must occur occasionally and the question therefore arises as to what role they may play in the production of effects on future generations, following an exposure to radiation. Under suitable conditions of selective multiplication, their role may be inordinately out of proportion to their relative numbers. So, for instance, in Gustafsson's (1947) extensive work with irradiated barley seeds only one mutation in about 800, on the average, was found to be of some use in adapting barley better for the needs of man, yet by discarding the other 799 and actively propagating the one advantageous mutant it was possible to establish an improved variety, and this procedure was in the end profitable. Of course the same sort of thing happens, in a slower and less regular fashion but on a far grander scale, in the natural evolution of organisms by spontaneous mutation, except that in this case the changes selected for multiplication are those which are advantageous for the species itself rather than for man. That is, despite the fact that the spontaneous mutations, like those produced by radiation, are in overwhelming majority detrimental, a kind of advance in adaptation nevertheless results by virtue of the selective multiplication of the very few gene mutations (and far fewer structural changes in chromosomes) that happen to be helpful. Thus it might be thought that the continual application of radiation would merely speed the advance, if other natural processes were allowed to take their course.

As a matter of fact this can and does happen under appropriate circumstances. Of the necessary conditions, the first is that the spontaneous mutation rate should not be already so high that when irradiation is applied mutations occur too frequently to allow an equilibrium elimination rate and/or a genetic load low enough to be tolerated by the population. A second condition is that the advantageous mutants should multiply fast enough to replace the original type at a rate commensurate with their increased rate of origination. A third requirement is that the organism should not be at the limit of an evolutionary blind end, i.e., that pathways of advantageous change still remain open to it. Such opportunities will be present in greater abundance, allowing more of the mutations that occur to be helpful in the given situation, if the population has been placed in an environment, and subjected to conditions of living, somewhat different from those previously natural to it; for it must already have become so highly adapted to its natural conditions as to make further progress difficult. Advance is also achieved more readily if the population is one which has to some extent lost, through genetic changes or recombinations, its original nicety of adaptation. This may have come about through the prior establishment of some more or less harmful mutations, the effects of which can now be overcome by reverse or counteracting mutations. Such prior retrogression is likely to have occurred if the given population has recently been

derived from one or from a mixture of a few more or less inbred lines, or from relatively few progenitors; in that case, moreover, the population will also start out with unusually restricted genetic variability, which the application of radiation will tend to remedy.

All the above conditions are fulfilled in certain recent experiments which have been carried out with *Drosophila* populations in independent work of Wallace (1951) and of Buzatti-Traverso (1951). In these experiments it was found that application of radiation to considerable laboratory populations, even for many successive generations, in doses that were rather heavy for this organism, resulted over a long period in a greater improvement of productivity of the flies, as measured under the laboratory conditions, than occurred in the nonirradiated control populations. These important but not surprising results are in harmony with the fact that a single pair of *Drosophila* will if given an opportunity produce hundreds of offspring, so that lines of descent derived from a relatively few flies of higher productivity are under some circumstances capable of displacing in a rather short time those derived from numerous others, of lower productivity. The free opportunity for breeding in the relatively large population cages used allowed intense natural selection of this sort, even though not so marked as this extreme example would suggest. Thus the high proportion of deleterious mutants could be kept from swamping the population, while those rare types which possessed any features that were advantageous under the given conditions outbred the original type.

Even so, it is very unlikely that a comparable improvement could have been brought about by these means in a natural population of *Drosophila* living under the conditions of nature. For one thing, these experimental populations must have had a restricted genetic background, as compared with natural populations, and this made a rise in the frequency of mutations more advantageous than it ordinarily would be. Second, there are many important features of the natural environment missing in even the best laboratory population cages (e.g., the relative inaccessibility of food in nature, and sometimes of mates; the incidence of drought, wind, predators and parasites; the existence of competing species and of other natural dangers), while on the other hand other features are present in the laboratory containers in more marked degree than usual. Under these changed conditions, opening new evolutionary pathways, not a few mutations would now be helpful which would have been disadvantageous in a state of nature, and the accumulation of these mutations might easily more than cancel the effect of an otherwise increased genetic load. In the course of the genetic reorganization process, retrogression in adaptation to the many features no longer of importance could occur with relative impunity, and those mutations furthering adaptation to the special laboratory conditions would still be advantageous even if they had

simultaneously entailed such retrogression. These factors, then, favored a relatively rapid advance in relation to the special conditions, despite a mutation rate that gave a load which under natural conditions might have placed the species at more disadvantage in relation to its competitors than before.

Nevertheless it is probable that the evolution of most species, even in a state of nature, would in the long run be accelerated by some increase in their mutation frequency, such as could be brought about by moderate doses of radiation. For, to follow up a suggestion made by Sturtevant (1937) and later developed further by the present writer (1950b), natural selection tends, where possible, to keep the mutation rate at a lower level than that conducive to their most rapid evolution. Granting this, however, natural evolution is usually extremely slow, especially in species which, like *Drosophila*, have for an extended period existed in a form much like their present one, as long as they continue to live under the same conditions as those which formed them. Thus even if a twentyfold increase in mutation rate, produced, say, by 3000 r per generation, did result in a twentyfold acceleration of their natural evolution when applied to them while living otherwise in a state of nature, it would be unreasonable to expect a perceptible change in them—much less a measurable increase in such over-all, already nicely adjusted characters as viability or fertility—in the course of, say, 50 years (1250 generations) of such treatments. For this would be equivalent to only a thousand years of their ordinary evolution, an insignificant period in most evolutionary history. It therefore seems certain that the seeming improvements noted in the laboratory populations represented entirely a reorientation to their new conditions of life.

The questions remain to be asked: at what cost to individuals did this accelerated reorientation take place, what would it have meant in human terms, and what would have happened in the case of a modern human population in which it had been attempted to practice irradiation similarly, although necessarily much more mildly, for many successive generations? In the first place, it should be recognized that even where, as above, the evolution consists of an adaptation to new conditions, the vast majority of the induced mutations, like the spontaneous ones, are detrimental to life and/or reproduction. Hence the increase of mutation rate entails a corresponding increase in the rate of elimination and in the genetic load; i.e., the price is unavoidably paid in what we have termed "frustrated lives." According to the estimates given above, it would take only some 100 to 300 r applied to all the human population every generation, to result, if it could continue until equilibrium between mutation and elimination rate were reached, in genetic death and completely frustrated lives for all but a minute fraction of the population in every generation. If, however, in addition to the increased elimination thereby

brought about there was to be, as in the flies, a natural replacement of the original type by multiplication of the very rare "superior" mutants, we should have to add the lives of numerous "normal" individuals, frustrated by that competition, to those of the individuals carrying an excessive load of detrimental mutations.

Of course nothing like so great an increase in elimination rate could be tolerated by any human population, still less by one living after the fashion of modern civilized communities. For the rate of human reproduction, especially under modern conditions, is so low that it would be quite impossible for a minute fraction of the population to multiply enough in each generation to make up for the loss of the remainder. In fact, even at present the populations of the technically most advanced countries are not much more than maintaining themselves. This is another way of saying that human beings are already near if not at or beyond the mutation rate which, in relation to their conditions of living and breeding, is the "critical" one. Nevertheless it might well be that a small increase in the mutation rate, such as that brought about by an average dose of only 20 r, could be tolerated. It would however take its proportionate toll in genetic deaths and load, as has been explained. At the same time, the relatively small increase in the potential speed of biological evolution which it would theoretically give rise to could not, under such circumstances as those in which modern man lives, redound to his actual improvement but rather to his deterioration.

The reason for this reverse influence of mutation rate on biological progress in the case of modern man is that he uses artificial means to counteract and in some important ways even to reverse the usual mode of operation of natural selection. Not only do modern mechanical and social devices for rendering living easier combine with medical methods to save for reproduction an increasingly large proportion of those who formerly would have met genetic death—a procedure which when not combined with eugenic ones tends greatly to raise the frequency of essentially detrimental genes—but the more distinctively human, or (to us) "higher" characteristics, of intelligence, foresight, and social behavior, in so far as they have genetic bases, are, according to the conclusions of the great majority of the serious students of this subject, actually at a disadvantage in reproduction, under modern conditions, in competition with their opposites. In "advanced" countries and classes it is on the whole the wiser, the more humane, and the more progressive who artificially restrict their families more, while those who have less foresight, less interest in the education and welfare of their children, greater clumsiness in techniques, more thoughtless profligacy and more superstitution, leave a larger retinue of offspring, whose lives are largely saved, by modern methods, to repeat the process. Not that such differences in behavior are exclusively or even mainly genetic in their basis, but they

must be so to some extent. Hence, in so far as selective multiplication occurs in regard to the genetic traits involved, it works in a direction antagonistic to the welfare and advancement of the population as a whole, a direction opposite to that taken under primitive conditions. There is evidence that this is occurring in technically advanced communities of all types, ranging from the U.S.S.R. to most democratic countries. This being the case, it follows that under conditions like our modern ones any increase in genetic variability, such as would be brought about by application of radiation, would hasten the degenerative process.

The implication is not intended that the social conditions and mores which form the basis for this reversed selection are inseparably bound with the possession and use of the techniques of modern civilization, and that there is therefore an inherent contradiction which will in the end defeat all men's efforts to better their existence. It is theoretically possible, by the voluntary exercise of conscious guidance over reproduction, and only by this means, to regain a beneficial direction for the process of selective multiplication in civilized man. Such a situation is not yet even in sight. If and when it should come about, it would be found that there was already in existence such a plentiful supply of natural variation in all human populations as would for a long time allow biological progress at as rapid a rate as the naturally slow multiplication rate of man would make feasible. Perhaps some day, still later, biological techniques of advanced kinds would make possible radically new forms of genetically selective multiplication, and then an artificial increase in variability could be taken advantage of without paying the higher price of an increased genetic load and increased elimination rate. That bridge cannot be crossed now, however, and it would not be justifiable to form present policies regarding irradiation on the assumption that such changes will come about. For the present, then, and with existing conditions in view, it is a fantastic and dangerous rationalization to imagine that an increase in the human mutation rate, brought about by current radiation practices, will further the biological improvement of mankind.

23. PRACTICAL APPLICATIONS OF THE ACTION OF RADIATION ON THE GENETIC MATERIAL

Although, for the reasons given above, it is not practicable to utilize the genetic effects of radiation on man for the purpose of improving his genes, nevertheless, if the conclusion presented on p. 388 be accepted, the chromosome changes caused by radiation, through their very destructiveness to proliferating cells, form the main basis of the great therapeutic usefulness of ionizing radiation. In this way malignant growths can be selectively injured and in some cases destroyed, and overdeveloped parts of some types (such as enlarged thyroids) can be reduced in their size

and/or activity. It is probable that a similar genetic mechanism is operative—this time on the foreign cells present—when radiation is used against certain parasitic invaders, notably fungi, when they are so superficially located as to be reached with a high enough dose without damaging too much human tissue. Sterilization by irradiation—another example of the same kind of effect—is, as earlier pointed out, inadvisable when it is intended to be temporary, and it is usually more surely and safely accomplished by other means when it is intended to be permanent.

There is, however, an interesting possible use to which sterilization by irradiation might be put, provided preliminary work along the given lines on the screw-worm fly *Callitroga americana* by Bushland and Hopkins (1951) is borne out in extensive field tests. The objective in such cases is the checking, and perhaps in given areas even the extermination, of certain noxious species (in the given case a parasitic species) which are subject to great seasonal variation in numbers. The principle of the method, which was suggested by E. F. Knipling, is to irradiate very heavily great masses of individuals, artificially bred on a vast scale for that purpose, and then to sow them widely throughout the breeding ground of the species during periods when the natural population, at its lowest ebb, is just about to multiply again. The irradiated individuals, being more numerous than the wild ones and mating with them, should tend to swamp out the latter's multiplication. A factor contributing to this result is that the spermatozoa of the recently irradiated males are still functional, so as to compete with normal sperm, but carry structurally changed chromosomes which cause the death of the great majority of zygotes resulting from the cross with untreated females, while the few offspring which do survive, although phenotypically normal, are nevertheless laden with structurally changed chromosomes which in turn kill off a large proportion of the zygotes in the next generation. By repeated large-scale application of this method to a population already depleted by it, it might be possible progressively to reduce its numbers, provided the foci of natural breeding are sufficiently accessible to be adequately reached in the process of distributing the irradiated individuals.

Another method of utilizing individuals which have suffered genetic damage from irradiation—this time probably in the form of gene mutation, however—for checking the ravages of those of the original type has often been proposed in the case of parasitic microorganisms. This involves the production of strains whose virulence has been so decreased that they may be used as live vaccines, in the manner of cowpox or BCG. The chief difficulty lies in obtaining varieties which can be relied upon never, despite their vast multiplication, to change back again via reverse or "suppressor" mutations to a virulent form. Doubtless a whole combination of mutations would be required for this. Tests for the presence of these, after the first mutation had already made the organisms non-

virulent, would usually be very difficult, except where various other recognizable traits were known to be regularly associated with different types of nonvirulence; such traits then could be successively superposed on one another.

Fortunately, such 100 per cent stability in regard to a given characteristic is not required in the case of other genetically desirable types of organisms. Thus the question arises, to what extent is radiation useful in the furtherance of the artificially directed evolution of organisms in the service of man? Certainly the main objection raised in the discussion of its proposed application for speeding human evolution does not apply with other organisms, since in their case one need have no moral compunction in producing innumerable inferior individuals and discarding them, if at the same time a few desired types arise. The chief questions then remaining are those of economy and of practicability in general.

Commercially valuable mutations in mushrooms affecting color, growth rate, and fruiting time have been produced by radiation (U.S. Atomic Energy Commission, 1952, p. 98). The experiences of Gustafsson with barley and other crop plants, mentioned on p. 436, showed that about 1 in 10 offspring of his irradiated seeds carried some definitely recognizable recessive mutation, and that of these mutations something of the order of 1 in 800 were useful to man in some way, as by increasing the yield under the conditions of cultivation or by causing the product to be better adapted for being gathered, processed, or consumed. Thus the plants had to be bred (and inbred) on a considerable scale, and a multiplication of about 8000-fold was necessary before the original numbers were reestablished, with the mutation now incorporated in the strain. With plants of the rapidly and comparatively inexpensively multiplying kinds here used, however, neither the time nor the expense of this operation was unduly great. It would probably have taken longer, and cost more, to find and to multiply, to an equivalent extent, the comparable mutants which must have been present, in far more scattered condition, in untreated populations of the same varieties. Moreover, in some such cases the chosen varieties may not yet be widely grown, and other varieties would probably have to be resorted to for finding the desired spontaneous mutants, if they could be found at all. The transfer of the mutant gene from these other varieties to the chosen one might then require a very lengthy process of backcrossing with the latter, in order to maintain all the desired features of its genetic complex. This roundabout procedure would in some cases be less economical than that involving irradiation of the chosen variety. The reader interested in further instances of the successful use of irradiation for the obtaining of improved varieties of plants of economic importance may be referred to the recent review by Gustafsson (1952), which cites many cases both in his own work and in that of others, carried out largely in Sweden.

Thus the decision as to whether the radiation technique will be helpful must depend upon a number of factors which differ according to the species and variety in question. In general, the larger, the slower growing, and the more expensive to raise a type of organism is, and the smaller the potential number of offspring per individual, the less suited it is to the use of the radiation technique for its improvement, since the individuals are less expendable. For the present this rules out mammals in most but not all cases. Furthermore, the more readily available large populations of the organism are, for a search for spontaneous mutations, the less advantage, other things being equal, is afforded by the application of radiation for mutagenesis. Where definite recessive mutations are wanted, in organisms which are mainly crossbred but can be readily inbred, these mutations, of spontaneous origin, can often be found with as high a frequency merely by inbreeding (and especially by selfing) as that with which equivalent mutations produced by radiation can be found. To match the fact that one irradiation may cause, say, 20 times as many mutations as arise spontaneously in one generation, the spontaneously arisen mutant genes have usually accumulated for a good many more than 20 generations, on the average, so that although they are seldom seen without inbreeding, they have nevertheless attained a correspondingly high frequency in the population. Inbreeding will then bring them to light. Moreover, it is sometimes true that the very mutations which are more desirable are more likely, through natural selection, to have attained a higher natural frequency.

Radiation is accordingly most likely to be found advantageous for small, rapidly multiplying organisms, including especially those which are habitually selfing (like many higher plants), or which have major haploid phases (as in Hymenoptera, Rotifera, and many microorganisms), and which have therefore been weeded comparatively free of spontaneous mutants. Where it is much easier for the breeder to obtain such organisms by cultivating them himself than by searching extensively for them abroad, the advantage of using radiation on them becomes emphasized. Where mutations are desired within restricted varieties that it is either impossible (e.g., because of their asexual nature) or impracticable (e.g., because of their complex of desired characteristics) to outcross, this circumstance constitutes another feature which favors the use of the irradiation technique.

Where the circumstances for irradiation are favorable, one is not necessarily confined to hunting, among the descendants of the treated organisms, for mutations which individually produce large, definitely recognizable effects, like most of the mutations dealt with in conventional genetic studies. Neither need the mutations be recessives. Thus, as the already cited work of Buzatti-Traverso on *Drosophila* has shown, the practice of ordinary selection, even without inbreeding, can, if inten-

sively and extensively applied, result in the accumulation of minor changes which collectively work in the desired direction, even though it would very seldom be possible by this means to obtain perceptible improvement in characteristics useful to the organism itself while it is living under exactly its natural conditions and retaining in other respects its original genetic constitution.

The above considerations suggest that microorganisms should on the whole present especially suitable material for the practical use of radiation for its mutagenic effects. There are already several examples to illustrate its use in this way. One is provided by experiments with the mold *Penicillium*, initiated by Hollaender (1945) and continued by a whole group of investigators. In this work, the irradiation was carried out in several successive steps. After each exposure that mutant was selected which had the best yield of penicillin and the colony derived from it was then used for the next exposure. In this way a strain was finally produced, incorporating all the mutations together, which had a yield some four to five times as high as that of the original variety. This result was of considerable economic and medical value. Somewhat similarly, Hollaender and his co-workers (Hollaender, 1945; Hollaender, Raper, and Coghill, 1945; Lockwood, Raper, Moyer, and Coghill, 1945; Raper, Coghill, and Hollaender, 1945), irradiating an already somewhat suitable variety of the mold *Aspergillus terreus* in order to obtain a strain with a higher yield of the economically important substance itaconic acid, were successful at the same time in increasing the concentration of it in the cell still further, and decreasing that of contaminating substances, even though they found, as expected, that the great majority of the mutations which affected itaconic acid production at all decreased the yield of it.

There would appear to be an enormous field still open in such work, especially when the wide variety and unlimited succession of steps possible in evolution are taken into consideration. So, for example, it should be possible by successive mutations to adapt bacteria, fungi, protozoa, and viruses to new hosts, or even to make free-living ones parasitic, as well as to increase their virulence for their hosts so as to make them useful in the control of insect parasites, predators, noxious weeds, and other inimical species. Tissue specificities also could be developed in parasitic microorganisms, such as have already been claimed to afford an attack on certain malignant tumors. In other cases, not destruction but a specific constructive influence on given host tissues—as in the production of useful galls—might be evolved. The opportunities of establishing, in organisms not now possessing them, other beneficial forms of symbiosis with microorganisms, such as already exist, for example, in ruminants and termites, seem so far to have been exploited only to a very limited degree.

But the possibilities are by no means confined to parasites and symbi-

onts. There is vast room for the further development of valuable or potentially valuable microorganisms for the production of foods, food accessories, and pharmaceuticals (e.g., cheeses, wines, antibiotics), and as food or fodder in themselves (e.g., yeast, plankton, algae), and also for use in varied biochemical reactions of economic importance in other ways, as in the production of innumerable industrially serviceable organic materials, and in the synthesis, by the efficient use of solar energy, of energy-rich combustibles. When the stupendous accomplishments of natural evolution are contemplated, and then the momentous changes even in comparatively long-lived species achieved by the trial and error methods of primitive man, possibilities like the above for microorganisms appear by no means too exaggerated to be in significant measure realized, even within the space of a few decades, by artificially accelerated and rationally guided evolution.

Although in such work other mutagens than radiation can be used, experience to date has indicated radiation to be the most satisfactory agent for this purpose, because of its convenience of application, its penetration, and the ready control of its intensity and timing.

24. IRRADIATION OF THE GENETIC MATERIAL AS A MEANS OF BIOLOGICAL INVESTIGATION

A primary purpose of the theoretician as distinguished from the biological engineer, in applying radiation to produce changes in the genetic material, is not merely to find given mutants or even to plot the incidence of the changes found, but to use his results as a means of investigating the mechanism whereby radiation brings about these alterations. Studies of this type will be discussed in Chap. 8. Other purposes of using this method of experimentation—interrelated with the above but primarily concerned with biological problems proper—are to throw light on the behavior, properties, and constitution of the genetic material itself, and to attack varied problems of evolution, development, physiology, pathology, and biochemistry in which genetics plays a role. It is not surprising that thus far genetics itself has been the subject which has been furthest advanced by this mode of attack, but the repercussions of these advances have been far reaching elsewhere. In fact, so successful has this tool proved in such work that only a cursory survey can be attempted here, indicating the general types of results thereby obtained in these fields.

24-1. *Field of Chromosome Behavior and Properties.* As one example of the contributions of radiation experiments to genetics, it may be pointed out that the whole mechanism whereby structural changes of chromosomes occur, whether as a result of irradiation or otherwise, has been worked out chiefly through the studies on their production by radi-

ation. These studies have established, among other things, the following previously unknown principles (which were in the preceding sections assumed to be true): (1) that the breakage of chromosomes occurs first; (2) that the unions of fragments happen as a later, quite separate step; (3) that broken chromosome ends have the property of being adhesive to one another and thereby becoming permanently united; (4) that originally free ends do not have this property and are therefore to be distinguished as "telomeres"; (5) that the adhesiveness does not manifest itself while the chromosomes are in a condensed stage but remains latent, to be expressed on their emergence therefrom; (6) that the reproduction of a chromosome fragment produced by breakage results in that end of the daughter piece which is homologous to the broken end of the mother piece being itself adhesive; (7) that when union of broken ends occurs it is always two-by-two, i.e., 3 or more pieces cannot join at one point so as to produce a branched chromosome; and (8) broken ends do not adhere to the side of a chromosome. Breakage, however caused, is the event that triggers the process, but what happens later in the production of a structural change follows in consequence of the operation of the other principles. For evidence on most of these points the reader is referred to the present writer's analysis of the processes (Muller, 1940a).

The establishment of the chromosomal interpretation of the ropelike bodies in Dipteran salivary glands—a step most serviceable for progress in genetics—involved the use of chromosomes structurally changed by radiation. Only when the very changes which genetic tests had demonstrated to have been produced by radiation in the linkage maps were directly seen in the salivary gland bodies was the chromosomal nature of the latter revealed unmistakably by Painter (1933, 1934). Then, after varied chromosomes that had been structurally changed by radiation had had their morphology determined by the genetic method of "mapping" through linkage tests, the detailed study of the appearance of these chromosomes in the salivary glands made it possible to ascertain exactly which spots on the visible salivary chromosomes corresponded with given points in the linkage maps. In this way verification was provided of the physical validity of even the minutiae of the linkage maps, much as such verification of their gross features had earlier been given by comparisons of the linkage maps of chromosomes structurally altered by radiation with the chromosomes as seen in their condensed (mitotic) stages (Muller, 1928b, d; Muller and Altenburg, 1928; Muller and Painter, 1929; Painter and Muller, 1929; Dobzhansky, 1929, 1930a, b, 1931, 1932). Moreover, after the point-by-point correspondences had been ascertained in this way for salivary-gland material, various further conclusions could be drawn, such as that the frequency of crossing over varies in given ways from region to region of a chromosome, owing to the influence of the centromere and other features, and that the condensed mitotic chromosome

has some of its parts (notably the blocks) very differently spaced from the extended (salivary or other interphase) chromosome.[9]

The wealth of chromosomes with parts variously rearranged by radiation was further amplified by genetic techniques whereby, through crossing between the different chromosomal mutants, still more diverse recombinations, having given parts missing or duplicated, were obtained. Through the cytogenetic study of the resulting material it was then possible to gain information concerning the properties of the different chromosome parts. In this way it was proved that the centromere is the product of a gene, or a minute group of genes, located at the same point as that at which the centromere itself appears, but capable of being altered in position by structural change along with the chromosome region surrounding it. It was likewise established that one centromere is necessary for a chromosome to be transported at cell division, but that more than one, even if they are close together, leads, in the material used, to loss of the chromosome, and that chromosome bridges caused by dicentric chromosomes in fertilized eggs usually result, in *Drosophila*, in the death of the zygotes. Similarly, evidence was obtained of the genetic permanence of the telomere and of its being due to a gene or a minute group of genes located at the point at which it appears.

Other differentiated chromosome parts that were shown to be produced in situ in *Drosophila* (i.e., by specific genes located at the points in the chromosome at which the given structures appear) and to be separable by structural change both from each other and from the centromere, are the blocks (bodies whose existence had not previously been suspected), the nucleolus, and minute regions containing what may be called "conjugator genes," which give those regions a powerful effect on chromosome conjugation and segregation. The specialized genes responsible for all three of these kinds of effects were found to be congregated together, along with the centromere, in a given chromosome region, the main heterochromatic region, which exhibits a whole complex of other characteristics. These include, as was shown by these investigations, heterologous conjugation, relative dispensability of genes, susceptibility to structural change, characteristic cytological appearance, and peculiar type of position effect. Studies of rearranged chromosomes showed that these characteristics also were all in situ results of the genes in the same regions, and that they were not to be attributed just to the specialized genes above mentioned, which formed but a small part of these regions,

[9] Except in certain special cases, where the sources of evidence are not well known even to many geneticists, references will not be given to the voluminous literature in which the production of genetic changes by radiation has been used primarily as a tool in the investigation of genetic or other biological problems themselves, since the main purpose of the present chapter is to acquaint the reader with the nature of the genetic effects of radiation, from the point of view of those interested in radiation effects as such.

but were manifestations of the genes of this region in general. Hence these characteristics became transferred in position when a part of the latter did even when the above specialized genes were not included. Smaller heterochromatic regions, showing most of the same peculiarities but to a more limited degree, were found to be present just proximal to the telomeres, and a part of the effects, still less well developed, were found to exist in scattered interstitial positions as well. All this work required the production of structural changes by radiation. For most of the points in this and the preceding paragraph the reader may be referred to Muller (1938, 1944), Muller and Gershenson (1935), Muller and Prokofyeva (1935a), Muller, Prokofyeva-Belgovskaya, and Raffel (1938) and Prokofyeva-Belgovskaya (1935, 1938, 1939).

It was mainly through observations on the mode of transmission of chromosomes changed by radiation, and on the phenotypic effects of different combinations of the parts of these chromosomes, that the principles were worked out which govern the manner and speed of elimination, retention, or multiplication of different types of structural changes in populations. In this way it was possible for the present writer (1940b) to make deductions concerning the role of different types of chromosome alterations in evolution. The conclusions thus arrived at were corroborated by observations of other investigators, who had studied the distribution of chromosome differences among natural populations of the same species, and among different races, subspecies, and species.

24-2. *Field of Gene Properties and Gene Evolution.* Study of the somatic effects produced in *Drosophila* by the addition or subtraction of given chromosome pieces, made possible by the use of structural changes obtained from irradiation experiments especially designed for the finding of aberrations appropriate for this purpose, has resulted in a considerable extension of knowledge concerning genic balance and related matters. For example, the subtraction of a given portion of a chromosome from the diploid set of chromosomes was found, in general, to cause a good deal more developmental and physiological disturbance than the addition of the same portion to it, in correspondence with the fact that the subtraction constitutes a greater relative change than the addition. Similarly, the amount of disturbance caused by a given addition or subtraction is smaller, the larger the number of complete chromosome sets (i.e. the degree of "ploidy") present in the individuals being compared.[10]

[10] It may well be because of this principle that so many dominant genetic abnormalities, involving complexes of characteristics, have been observed in the first generation of offspring of irradiated salmon spermatozoa, in the work of Foster, Donaldson, *et al.* (1949 and personal communication). There is reason to believe these fishes to be of polyploid origin. Most of the abnormalities would in that case be caused by extra or missing whole chromosomes or large chromosome pieces, which would have been lethal to ordinary diploids, and the apparent contradiction between these results and those on most organisms would thus be reconciled.

Cases involving subtraction of pieces, "deficiency," further showed that there are only a limited number of individual genes—probably several score altogether, scattered along the chromosomes—the reduction of which from two doses to one results in full lethality or in a definite, readily observable morphological effect. Moreover, a considerable proportion of the morphological effects in these cases consists in a given, regularly appearing syndrome (the "Minute bristle" complex of characteristics). But, apart from these marked effects of a few genes, it is in general true that the larger the deficiency, in any chromosome region, the lower the viability and fertility, until a size of deficiency is reached which cannot be tolerated. This maximum size varies with the region but is never greater in *Drosophila* than a few per cent of a total chromosome-set (as in the case of the loss of an entire fourth chromosome), and is usually less than 1 per cent. This result is necessarily due to the cumulative action of very many individually small effects, i.e., to the collective weight of numberless fractional genetic loads, each caused by the heterozygous (single dose) state of a normal gene (compare discussion in Sect. 20).

It was observations of the consequences of changing the dosage of individual mutant genes of known morphological expression, effected by the addition or subtraction of small chromosome fragments produced by radiation and chosen so as to contain these genes, which gave the most significant results concerning the phenomena of genic balance. This was the work which provided the evidence for the classification of mutant genes presented in Sect. 13, and for the finding that the majority of detected mutants are hypomorphs, with amorphs second in frequency. It was this work which at the same time showed that for most genes the phenotypic effect at first (for hypomorphs) rises steeply with increase of gene activity or dose, and that the curve of effect (Fig. 7-6) then becomes convex, approaching a saturation level, which, however (as the studies on dosage compensation showed), is never fully attained. This furnished a simultaneous, common interpretation based on evolutionary and biochemical considerations for (1) the phenotypic variability of most mutants, (2) the phenotypic stability of the normal type, (3) the fact that most normal genes appear to have the same expression when present in two doses as when present in one, and (4) the dominance, in most cases appearing complete when judged by ordinary inspection, of most normal genes over their mutant alleles.

Through the same tests, the facts of dosage compensation also came to light, and this phenomenon was further investigated by studies utilizing a systematic series of radiation-induced chromosome fragments. Thereby cogent evidence, quite apart from that already mentioned, was obtained that despite the seemingly complete dominance of normal genes there must usually be enough difference between individuals with two and

those with one dose of a normal gene, and hence, too, between homozygous and heterozygous normals, to affect their genetic survival significantly—so significantly, in fact, as to have led to the establishment of systems of dosage-compensating genes. This then demonstrated the importance for the organism of shades of difference so minute as to be below the threshold for ordinary detection, and showed that these subliminal effects have been accumulated in the course of natural selection until a remarkably high degree of precision of genetic adaptation has been attained by the normal type.

The conclusion, thus doubly arrived at through radiation studies, that the dominance of normal genes is not actually complete, was later verified, by the direct measurements referred to in Sect. 14, of the viability of individuals heterozygous for lethal and sublethal genes that had been produced in radiation experiments. These showed the amount of expression of the "recessive" mutant genes in the heterozygote to be sufficient to result in mutant genes being eliminated while in the heterozygous condition, in the great majority of cases. Since the evidence of Levit derived from spontaneous mutations in man fitted in with this, an entire reordering of ideas and recalculation of results pertaining to rates and curves of elimination, types of expression, and equilibrium frequencies of mutant genes—whether spontaneous or induced—in populations became necessary. The application of these methods to the actual situation also required the use of another contribution of radiation genetics, in which light had been thrown on the relative frequencies of mutations having different types and degrees of expression: visibles, detrimentals, steriles, and lethals, and in which estimates had thereby been arrived at of the total frequency of mutations. In the process of combining the results from the two fields of investigation, on the degree of dominance and on the frequencies of mutations, respectively, the concept of genetic load had to be introduced. It proved a fruitful one in assessing the effects of radiation and of selection on populations and on the individuals composing them.

Studies of chromosome changes produced by radiation threw light from still different angles on the properties of genes. For example, it was the finding of the regularity with which, in *Drosophila*, structural changes are accompanied by detectable phenotypic effects, such as lethality, sterility, and morphological abnormalities, that suggested the conception of position effect as a general, fundamental phenomenon (even though not evident in most organisms), rather than one confined to special cases. Numerous subsequent studies, employing chromosomes changed structurally by radiation in various ways, verified this idea and disclosed important additional features, such as the peculiarities of the position effects resulting from the juxtaposition of eu- and heterochromatic

regions. Nevertheless, the problem of the physicochemical nature of position effects is still an unsettled one.

Another series of studies in which chromosome changes induced by radiation have been utilized for the light they throw on gene properties has dealt with the number of genetically separable positions of breakage which exist within a given minute chromosome region, measurable in salivary preparations (Muller and Prokofyeva, 1935b). In this work a series of structural changes, all selected, through their position effects, to have one chromosome break located very near a certain gene ("scute"), were genetically cross-tested with one another by special methods which made it possible to determine the positional order of the breaks in the chromosome, from left to right. This involved getting recombinations between the different cases of structural change, having the left-hand portion of one changed chromosome (A) extending up to its break in this region, together with the right-hand portion of another (B) extending rightwards from its break, to discover whether the AB combination was lethal or exhibited any other phenotypic abnormality indicative of a deficiency. The complementary BA combinations, having the left part of B with the right part of A, were obtained and examined similarly. When AB proved deficient break A was shown to be to the left of break B, while when BA was deficient break B was to the left of A; but when neither recombination behaved as a deficient one, it could be concluded that the breaks were at identical positions, in the sense that no genetic material having a detectable influence on the organism lay between them.

It turned out that some dozen cases of breakage in the given region involved only four positions of breakage, as thus determined, and from certain additional evidence just one more possible position of breakage was deducible. Each two breakages thereby defined, lying in consecutive positions, enclosed between them a gene with distinguishable effects. The evidence thus indicated that the chromosomes become broken only in certain discrete positions, between genes, and that the genes are to be regarded as distinct entities. It may be recalled that evidence for the same conclusion was also given by the finding that, in most organisms, which show little or no position effect, structural changes are very seldom accompanied by lethal effects or other phenotypic abnormalities.

A study somewhat similar to the above in principle although not in technique has been made in maize by McClintock (1938b, 1941, 1944). Here chromosomes structurally altered by X rays were used which have a sequence of breakage-fusion-bridge cycles, resulting (when homozygous or when in combination with certain other chromosomes which had also been structurally altered by radiation) in plants and parts of plants wholly deficient for a small chromosome region. Two different chromosomes (5 and 9) were used in different series of experiments. The limits

of the deficient region differed slightly in the different cases of any one series of experiments in consequence of minute differences in the positions of breakage. By noting the phenotypic effects produced in the different cases, and by comparisons of the manner of grouping of these effects from case to case, the linear sequence and the functions of the genes contained in the affected portion of the chromosome could be ascertained with great nicety of resolution, yet over a considerably greater length of chromosome than in the work with the scute region of *Drosophila*.

Returning now to the studies on the scute region, a deficiency that according to genetic tests lacked exactly that portion of chromosome lying between the leftmost and the rightmost of all the breaks which had been dealt with, and thus was deficient for the whole group of four adjacent genes comprised in the preceding analysis, was found on cytological examination to occupy a length of about half a micron on the salivary chromosome and to constitute not more than a half of one double band as seen in ordinary preparations. In this way it was shown that the maximum length of the individual genes here dealt with was just beyond the resolving power of visible light, even in the salivary chromosomes, and occupied less than one ordinary band. From the total length of a complete set of salivary chromosomes it could then be readily reckoned that there would be room for some 8000 such genes, if they were similarly spaced throughout, while if they were contained only in the chromatic regions there would be some 3000. These estimates of gene number, divided into the size of one complete set of chromosomes when it is most condensed (as at mitosis or in spermatozoa), gave maximum estimates for gene volume (Muller, 1935a). The approximation figures for gene number thus arrived at (and therefore also those for size) proved to be in satisfactory agreement with estimates of gene number (and size) obtained by two quite independent methods, which involved a larger risk of error. One of these methods was based on the minimum "map distance" found between genes in representative portions of the genetic linkage diagrams. The other was based on the frequencies with which gene mutations recurred in the same locus, as compared with the frequencies of gene mutations in different loci. In some of the applications of the latter method, also, radiation had been used for the production of the gene mutations studied.

Radiation genetics has provided evidence not only concerning the manner of subdivision of the genetic material along the chromosome, i.e., in a longitudinal direction, but also concerning its possible compoundness in a transverse direction. In fact, the obtaining of evidence on this question, at a time when it was thought that the gene might be composed of several or many identical units, termed "genomeres" (Muller, 1926b, formed one of the principal motives for the present writer's first work on the production of mutations by radiation (1927, 1928a, b, d). For if,

as seems most likely, a mutational change involves only a single genetic element at a time, then if the gene before its mutation is compounded of several identical parts, one of which mutates, it should follow that successive reproductions of the parts and their distribution at the consequent mitoses will result in a mosaic of cells, some of which will contain elements of that gene all of which are mutant and others elements all of which are normal. In contrast to this, no such patchwork of mutant and normal tissues could be found in the case of visibly expressed mutations, in individuals derived from irradiated spermatozoa. Nor did there seem to be any such long-delayed production of stable mutations (representing genes which had come to receive elements all of which were mutant) as this view would call for. It was accordingly concluded that the entire gene constituted a unitary (though complex) rather than a compound organization, and that the genetic material of the chromosome thread, in the *Drosophila* spermatozoon at least, was probably single.

Perhaps the most important finding in the work on the production of gene mutations by radiation, as far as its bearing on theoretical genetics, evolution study, and general biology is concerned, is that, as pointed out on pp. 394–395, the mutations bear so much resemblance to those which occur spontaneously, and have such a similar even though not always identical distribution and relative incidence of the different phenotypic effects. Since the production by radiation of one rather than another mutation on any given occasion must have been determined by factors involving the physical distribution of ionizations or excitations, and since these events must have been accidental, in the sense of being unregulated by the organism itself, it became reasonable to conclude that the spontaneous mutations, inasmuch as they so resemble those produced by radiation both in types and manner of incidence, must be similarly accidental in their origination, rather than representing any sort of adaptive biological response to given conditions. In this way the radiation results provided significant support to the theory of fortuitous genetic variation, which has as its corollary the conclusion that natural selection constitutes the guiding factor in the genesis of adaptations, and hence in biological evolution in general, as Darwin proposed (Muller, 1929, 1947).

In many cases radiation has been used for the purpose of furnishing mutant genes to serve as "markers" in the making of genetic maps, for such maps, together with the stocks containing the genes shown in them, then prove useful in further genetic studies of varied kinds. In several such instances the investigator has thereby been put into a position in which it was possible for him, in the given organism, to establish new principles of genetics, which the other organisms used in genetic work were not adapted to reveal. One case of this kind is the elucidation, by P. W. Whiting (1940, 1943), of a hitherto unknown mechanism of sex determination, that in *Habrobracon* and probably in various other

Hymenoptera. In *Habrobracon* the addition of radiation-induced gene mutations to the spontaneous ones allowed the genetic "marking" of all the chromosomes. This provided a means of proving that only one of the chromosomes of any set, and its homologue in other sets, was sex-determining. More intensive study of this chromosome then established the fact that it existed not in two but in multiple forms, any two of which together resulted, by a complementary action, in the female sex, whereas one kind by itself resulted in the male. Another case is that of the working out of the system of Mendelian, chromosomal heredity present in *E. coli*, by Lederberg (1947) and Lederberg *et al.* (1952), through studies of the linkage relations of mutant genes most of which were produced by radiation. This in turn has served as the necessary basis for the establishment in these bacteria of a number of important genetic principles, of a hitherto unique type. In other cases such work has facilitated genetic comparisons between species, for the purpose of determining the types of changes undergone by them in their evolutionary divergence from common ancestors.

The utility of radiation for making surveys of the distribution of gene mutations in the germ plasm as a whole, and also for the intensive study of the mutational potentialities of individual genes, is obvious. Work of both these types has thus far been carried furthest in *Drosophila* and maize, but results along both lines in *Neurospora* and mice are also becoming impressive. It would require too much of a digression even to summarize them here, though with regard to the first line of attack reference may again be made to the studies on the relative frequencies of different phenotypic classes of mutations (e.g., lethals, detrimentals, visibles). As for the intensive studies of individual loci, the most detailed work, such as that on the scute, white, dumpy, lozenge, bithorax, and Stubble loci or groups of loci in *Drosophila* and on the A locus in maize, have demonstrated the high complexity of some of these genes, as evidenced by the number of different alleles they could form and the diverse directions the mutations of one gene could take. The same work also showed that different parts or operational features may undergo alteration separately from one another in some mutations, and together in other mutations of the same locus or group of loci. This was true both of radiation-induced and spontaneous mutations. The qualification, mentioned on p. 411, should here be repeated, however, that ionizing radiation in maize (unlike ultraviolet in maize and *Drosophila*, and unlike ionizing radiation itself in *Drosophila*) seemed to give only complete deficiencies of one or more loci, when a given locus (A) was chosen for observation (Stadler, 1941; Stadler and Roman, 1948).

The term "group of loci" was used advisedly in the foregoing paragraph since some of the induced mutations at first thought to be allelic, in the sense of consisting of changes of the same gene, proved to be muta-

tions, similar in their effects, of separate but very closely neighboring genes. Although these have usually been interpreted as "duplicate loci," descended from a common ancestral locus that had undergone a duplication which became established in the evolution of the normal type, it seems probable that some of them are cases in which two essentially different but neighboring genes interact through a position effect. However, in one case at least, that of "scute" and its neighbor gene "achaete," it was possible to prove by means of radiation-induced structural changes that the two loci are able to exert most of their characteristic effects even when they are widely separated from one another. In this case then the evidence of their origination by duplication is convincing. The present-day difference in their normal function, and in their mutational potentialities, illustrates well the mutational differentiations which such duplicated loci tend to undergo in the course of their evolution subsequent to the duplication. Another very instructive example of this kind is furnished by the loci for sperm motility present in the Y chromosome. It was shown in ingenious work of Neuhaus (1939), utilizing the position effects of a large series of radiation-induced translocations involving breaks at slightly different positions in the Y chromosome, that there are over a dozen different but related genes in that chromosome, the combined action of all of which is necessary for sperm motility. These genes must have arisen through repeated duplications of a common ancestral gene, and after duplication have undergone mutually complementary mutational differentiations.

It was also proved by fragmentation of the X chromosome of *Drosophila*, brought about by application of X rays, that this chromosome contains a considerable number of loci which act cumulatively in sex determination (Muller and Stone, 1930; Patterson, 1931a; Dobzhansky and Schultz, 1931, 1934; Muller, 1932a; Patterson, Stone, and Bedichek, 1937). It can, however, be inferred on evolutionary grounds that there was at first only one such locus. The present multiple condition could hardly have arisen exclusively by duplication of that locus, since this, at the first such step, would have given one X chromosome the potency of two and so would have upset the whole sex-determining mechanism. It is therefore necessary to conclude that there was, to some extent, a dispersal of the sex-determining function over a number of different genes, by means of gene mutations in them (Muller, 1939a); the original sex-determining gene must meanwhile have diminished in its potency, in a number of steps. Thus, as with the sperm motility gene of the Y chromosome, but by a mechanism to some extent different, the entire collection of these genes finally became necessary in order to fulfill completely the function originally carried out by one gene.

An example of a very different method whereby the production of chromosome breakage by radiation has made it possible to obtain evi-

dence concerning what has happened to genes in the course of their past evolution is furnished by studies by the present writer and Pontecorvo (Muller and Pontecorvo, 1940, 1942a) of the effects of substituting given chromosomes of *Drosophila simulans* in the place of their *D. melanogaster* homologues, in an otherwise *D. melanogaster* genotype. Natural hybrids between these two species are always sterile. Hence the species are essentially unmixable, in the sense that natural recombinants between them cannot be obtained, and the gene differences between them would therefore appear to be unanalyzable. However, it was found possible to circumvent this difficulty by heavily irradiating *D. simulans* males and then crossing them to triploid *D. melanogaster* females, all of whose pairs of chromosomes had been made homozygous for recessive "marker" genes. In this way zygotes could be formed, and recognized by their markers, in which one or more chromosome-pairs were species-heterozygotes and the rest homozygously of *melanogaster* origin, just as if these zygotes had been second-generation individuals that had arisen through the impossible cross of a sterile hybrid male back to a marked *melanogaster* female. The homozygous *melanogaster* pairs of chromosomes in such individuals arose from the fact that the egg had in these cases received two of these chromosomes from the triploid mother while the homologous *simulans* chromosome of the sperm had been broken by radiation and lost by the fusion-bridge sequence.

By noting which recombination types survived and what characteristics they had, various deductions could then be made concerning the roles played by the genes in different chromosomes in the production of hybrid sterility, low viability, and morphological abnormalities. It was at the same time proved that the species differences responsible for all these effects had their genetic bases located in the chromosomes, there being no cytoplasmically located genetic residuum for the given effects. Moreover, through the lucky case of a fertile hybrid, all of whose major chromosomes had been derived from *melanogaster*, the transfer of the small fourth chromosome into an otherwise *melanogaster* stock was accomplished. Through the more intensive study of this "introgressive hybrid" stock, the prevalence of interspecific gene differences more cryptic in their expression than those dealt with above, but also chromosomal in their location, was then demonstrated.

The above experiments represent, in a way, a further extension of the technique used in those investigations—ranging from the early ones of the Hertwigs (e.g., G. Hertwig, 1911; P. Hertwig, 1917) to the recent ones of A. R. Whiting (1948)—in which the loss of the entire complement of chromosomes in the sperm or egg, induced by heavy irradiation, has resulted in genetic uniparentalism of the maternal and paternal types, respectively, according to whether male or female gametes had been treated. These effects have been produced in both intra- and inter-

specific matings. They allow deductions regarding the time and manner of onset, in the embryo, of effects traceable to the genes introduced by spermatozoa, and regarding the interaction of these with the stored gene products derived from the chromosomes which had been present in the egg and in the surrounding soma before fertilization.

This account far from exhausts the varied ways in which the production of genetic changes by radiation has already been used for throwing light on questions of genetics and evolution. Thus, in the *Drosophila* work, both the gene mutations and structural changes so obtained have vastly increased the number and diversity of genetic tools available for the attack on problems of the most diverse types. In many cases these tools have been constructed to order. For, even though no control can be exercised over the type of mutation that will occur in any given germ cell, nevertheless mutations can be produced in such abundance that, with a suitable genetic setup for the detection of given, desired types of mutants, it is often practicable to carry through operations expressly designed for finding changes of these particular types. The latter can then be recombined, by crossing, into a variety of arrangements, useful for diverse types of investigation. Similarly, in maize, Anderson and Randolph (1945) have produced translocations by irradiation and thereby "tagged" given chromosomes in order to follow and control the distribution of genes useful in practical breeding and in investigation. In silkworms, Tazima (in press) has by inducing deletions elucidated sex determination.

Many important stocks of *Drosophila* contain recessive lethal, near-lethal or sterilizing genes or gene combinations which it is desired to preserve. Since these cannot be bred as homozygotes, continual selection of the appropriate individuals would be required, unless there was a genetic "balancing" arrangement present which resulted in the death or sterilization of individuals *not* carrying the desired gene or genes as well as of those homozygous for them. This balancing is accomplished by the introduction of one or more lethals or steriles into the chromosome homologous to that carrying the desired gene or genes, and it is usually necessary also to have one or more inversions present, heterozygously, which will effectively prevent crossover individuals, free from the lethals or steriles, from being produced. Varied "balancing chromosomes," equipped with the required lethals or steriles and also with suitable inversions, have been provided by irradiation, some of them in work designed for obtaining them. Thus the maintenance of *Drosophila* stocks of most of the desired types has been rendered automatic, in the sense that no artificial selection is required, and the number of stocks which it is feasible to keep has thereby been greatly increased.

In addition to their use in the mere maintenance of stocks, such "balancing chromosomes" of *Drosophila*, most of which owe their origin in part at least to radiation, are increasingly being put together in combina-

tions, some of them very elaborate, which provide precisely designed genetic machinery, of varied types, for use in the more or less automatic carrying out of given genetic operations that require repetition on a mass scale. For example, in the finding of lethal and other mutations in the second chromosome, a task which requires breeding as far as the third filial generation, the chief factor which in the past limited the scale on which any such work could be carried out was the necessity for obtaining virgin females of a particular kind from each of the numerous second-generation cultures, for breeding with males from the same culture, so selected as to have second chromosomes of the same kind as those in the females. But nowadays, by the use of a technique involving a specially constructed "sifter stock" (Muller, 1951b), in the production of which radiation was employed but which is too complicated in genetic structure and operation to be described here, all flies of the second filial generation meet with genetic death before maturity except the females and males of the required kind. Thus the females do not need to be obtained as virgins, and the offspring of this generation need merely to be transferred en masse to new cultures, for the production of the third generation; in the latter generation the presence of the mutations being sought for is readily evident on inspection. In such ways, then, the genetic tools provided by radiation have greatly increased the productivity of a given amount of work, especially in the fields of mutation frequency and of the frequency of mutant genes in populations. At the same time they have made it possible to employ, in part of that work, less highly skilled assistants than were formerly necessary for it.

24-3. *Fields of Development, Physiology, Pathology, and Biochemistry.* Not only problems of genetics proper and of evolution, but also those of development, have had light thrown upon them by making use of the genetic effects of radiation. One such line of attack is concerned with the tracing of the cell lineage of parts of the body, and with the degree of autonomy with which given characters develop. This is well illustrated in Patterson's (1929) experiments in irradiating the embryos and larvae of *Drosophila* which were heterozygous for the recessive gene for white eye. In these experiments observations of the size, shape and position of the resulting white spots in the eyes of the adult flies—spots now known to have been produced, in the great majority of cases, by somatic crossing over (Muller, 1941; Auerbach, 1945)—showed that the cells of the optic anlagen divide approximately once in 12 hours, up to a given stage. The observations showed, further, that the region of the eye which a given cell is to form is indeterminate except that the descendant cells tend to remain together in a group, and that the pigment develops autonomously in this case, i.e., its development or nondevelopment is determined by whether or not the given cell contains the normal allele of white, regardless of which allele the neighbor cells contain. Similar work, involving

other parts of the body, has been carried out with other characters, including those associated with a difference in sex. In some cases the technique has involved the breakage and loss of an entire chromosome by treatment either before or after fertilization; ring chromosomes are especially suitable for this purpose.

Inasmuch as the inhibition, by radiation, of those processes of growth, differentiation, and regeneration which require cell division is probably caused by the damaging action of structural changes of chromosomes on the cells descended from the irradiated ones (as noted in Sect. 10), any morphogenetic or other developmental studies employing radiation in this capacity constitute illustrations of one type of use to which the genetic effects of radiation are put for the investigation of developmental processes. This method has proved a fruitful one in the hands of experimental embryologists, especially when the application of the radiation has been limited in space and time to certain parts and stages (e.g. blastemas), whose influence on a given developmental reaction can thereby be traced. The method is likewise useful in the study of some physiological processes of the adult which depend upon the proliferation of given cells (e.g., those of the hematopoietic system), since it makes possible the study of the consequences of reducing the effective numbers of these cells.

Potentially by far the most analytical use to which the production of genetic changes by radiation may be put in studies of developmental processes is through its provision of mutant genes, the effects of which on development are then traced in detail. A great many studies of the ways in which given mutant genes influence development—a field of ingestigation known as "developmental genetics"—have been carried out in *Drosophila* and other small organisms commonly used in genetic work, and a few, of considerable interest, in mice and poultry. Although hitherto genes which arose by spontaneous mutation have usually been employed, it is to be expected that, with the increasing use of radiation to produce mutations, in higher as well as in lower forms, the genes which are obtained in this way will furnish an ever larger portion of the material for such work. The field is a virtually unlimited one since, theoretically, the method could be applied for each of the thousands of different genes capable of mutating, and even for each of the different mutant alleles of these genes. Moreover, it can be used for gene combinations, in the study of the effects of gene interactions, as well as in combination with varied environmental conditions and artificial techniques. In fact, in the face of this overwhelming wealth of possibilities, the greatest problem may be the proper selection of those types of gene effect for study which involve the more basic and significant processes. For although a complete knowledge of developmental reactions requires the eventual study of all gene effects, it is evident that the study of most of these

effects must remain on a superficial level until the outlines of the more underlying developmental reactions have been brought to light. In our ignorance of the latter, most present attempts along these lines are necessarily exploratory in character and their results must long remain disconnected.

Developmental reactions are of course physiological, in the broader and at the same time more accurate meaning of that term. Moreover, the recognized physiological processes of the adult are resultants of developmental ones which preceded them, and some of which are still necessary for their maintenance, or for their gradual change in the course of aging. Thus the activities with which the physiologist deals are as much dependent, in the end, upon genes and their interactions as are those studied by the embryologist, and they are similarly susceptible to analysis through the intensive investigation of the processes in question in individuals having given mutant genes, and the comparison of these results with the corresponding ones obtained in normal or other genetically contrasting individuals. Illustrations of some well-known studies of this kind in man are to be found in the investigations of myasthenia gravis, hemophilia, and pancreatic fibrosis. Such work helps to elucidate not merely the pathological processes themselves but, as the other side of the medal, the normal mechanisms which are in these cases deranged. Although these hereditary conditions in man arose, of course, by spontaneous mutation, it is to be expected that, in laboratory organisms, the induction of such changes by radiation will play an increasing role here just as in the more strictly "developmental" studies. In fact, in one field of pathology, the study of tumors, radiation has already been found useful for obtaining, in *Drosophila*, genes giving rise to varied kinds of tumorous growths, which have provided material for the study of the development of such structures under different conditions.

Underlying and participating in all developmental and physiological processes, as well as all pathological ones, are biochemical reactions, and it is of course these which constitute the most fundamental field of operations for the investigation of the more proximate effects of genes—those effects on which and out of which all phenomena dealt with by the biologist proper and by the medical man are built. In other words, whether a given activity of an organism is called biochemical or not merely depends on the kind of equipment by which and the level of analysis on which it is being regarded. To quote an earlier statement by the present writer (1933):

> . . . each gene must be considered as producing its own specific chemical material in the cell, as distinctive in its composition as insulin or thyroxin are, . . . even though most of these materials do not circulate through the blood as hormones, and have not been extracted, but remain within the cells in which they are produced by the activities of the genes. It is a task for the future to deter-

mine the composition of all these substances and the nature of the complicated interactions whereby they cooperate to make the organism what it is. . . . It is evident that one chief method of attack in this new type of physiologic analysis, a method which must be of high eventual importance to pathology, as well as to physiology and embryology, would be the alteration or the excision of individual genes, one at a time, out of these thousands of genes, followed by intensive embryologic, physiologic and physicochemical study of the effects thereby produced on the organism. In other words, if we had the ability to change individual genes we should have, in effect, a scalpel or an injecting needle of ultramicroscopic nicety, wherewith to conduct the most refined kind of vivisection or biochemical experiments on our experimental animals, not experiments in which gross parts are removed, injected, or otherwise changed, but experiments in which the finest, most fundamental elements of the body fabric are separately attacked. . . . Changes in the genes which have arisen spontaneously and are already at hand can of course be used in such a study, but many of the most instructive types of these have already been largely weeded out by a process of . . . natural selection, before we can find the individuals containing them, while many of those still existing lie scattered far apart and concealed. . . . Hence the question of the production of changes in the hereditary material by means of roentgen or radium rays becomes all the more urgent.

Since the time when this was written, the method of utilizing the differences of reaction provided by mutant genes has had its greatest success at the biochemical level of investigation, and it is here that genes intentionally produced by irradiation have been employed most extensively for gaining further insights into the nature of some of the basic processes occurring in the protoplasm of organisms. Before the rise of the radiation technique and to a lesser extent afterwards, spontaneous mutations were employed in such studies. Examples are the work of Scott-Moncrieff, Robinson, and others, on the biochemical steps involved in the synthesis of flower pigments, that of Ephrussi and Beadle, of Kühn, and of others on the synthesis of insect eye pigments, studies of Onslow and of a number of other investigators on the biochemical genetics of mammalian coat color, and observations of Penrose, Garrod, and other medical geneticists on the group of oxidative processes in man which are affected in hereditary cases of such conditions as phenylpyruvic amentia and alkaptonuria. But since the employment of radiation in genetics has become more widespread, the attack along these lines has been greatly extended and facilitated by the aid of the mutations thus produced. It is natural that, thus far, the work with the radiation mutations has been carried on mainly with microorganisms, since, as noted previously, this is the type of material which yields returns along such lines most quickly and economically.

This is not the place to review the important results obtained by the application of this mode of investigation to the mold *Neurospora* by Beadle, Tatum, Horowitz, Bonner, and their associates, the pioneers in

this field, or to other fungi and to bacteria by numerous other workers. Until now, the methods used by them for detecting "biochemical mutants" have focused attention chiefly on gene changes which affect the ability of the given organisms (which are in most respects "autotrophic") to synthesize those organic substances which in most animals must be supplied ready made, such as amino acids, vitamins, purines, and pyrimidines. Considerable advances have thereby been made already in tracing the complicated courses of synthesis of these materials, despite the fact that the view of each enzyme being the product of one particular gene is proving to have been a serious oversimplification (Bonner, 1952), just as was the view of some early geneticists that all "characters" whatsoever of the organism bear a one-to-one relation to their genes. Such an interpretation is entirely unnecessary to the dramatic success which the method has achieved in unraveling biochemical reaction chains and networks, and not only cell physiology but even organic chemistry proper is falling in debt to this work.

Even at that, the possibilities of analysis of the metabolic processes common to higher organisms in general—including the holozoic ones—have as yet scarcely been scratched. They await the devising of methods of detecting, preserving despite themselves, and studying the biochemical effects of those still more numerous lethal and detrimental genes which have to do, not with the synthesis of the so-called "food constituents," but with the carrying on of anabolism and catabolism from that point forward. And beyond these more general and widely distributed biochemical reactions, in turn, lie the vast multitude of more special ones which serve in those processes of development, differentiation, and maintenance whereby each phylum, class, and even species is distinguished from the others. Here the studies of biochemistry, physiology, morphogenesis, and evolution meet. Here the subtlest tool of genetics—gene mutation—must constitute the major as well as the most delicate instrument. And in the provision of these gene mutations will be found the most important contribution which radiation can make in the solution of the problems of the biochemist and the biologist proper, as distinguished from those of the geneticist himself.

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