1928),[3] to the reversal of the ciliary stroke on the lips of Metridium. In consequence of the relative abundance of glycogen in the meat of mussels such a response might have been anticipated. No reversal was obtained from ordinary starch or from glucose when these substances were applied to the lips of the sea-anemone as the glycogen was. I conclude that glycogen may be added to the short list of substances by which ciliary reversal in sea-anemones may be induced.

[1] Parker, G. H., 1905*a*. "The Reversal of Ciliary Movement in Metazoans," *Amer. J. Physiol.*, **13**, 1.

[2] Parker, G. H., 1905*b*. "The Reversal of the Effective Stroke of the Labial Cilia of Sea-Anemones by Organic Substances," *Amer. J. Physiol.*, **14**, 1.

[3] Parker, G. H., and A. P. Marks, 1928. "Ciliary Reversal in the Sea-Anemone Metridium," in press.

THE PRODUCTION OF MUTATIONS BY X-RAYS[1]

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Read before the Academy April 24, 1928

1. *Earlier Experiments—Magnitude of the Effect.*—In the earlier experiments on this subject, which may first be referred to briefly, crosses were made between flies containing a different collection of genes in their X-chromosomes (the males having bobbed, and the females scute, vermilion, forked). In most cases only one parent was subjected to the X-ray treatment. The female offspring from these crosses would contain any new mutant gene that might have arisen in only one of their X-chromosomes, and hence they would manifest no abnormality if, as usual, the mutant gene were recessive. By breeding them, however (they were bred to their brothers), males would be produced in the next generation that manifested any visible mutation occurring in their X, or, if the mutation were lethal, certain expected classes of males would then fail to appear. The counts would also show in which chromosome—that from the original male or female—the mutation lay, and at approximately what locus in that chromosome it lay. And since, owing to the nature of the cross, some flies of the same composition as those of the first offspring generation were again produced in the second offspring generation, the same cross could then be repeated, and repeated again, in order to determine whether further mutations continued to originate in later generations, long after treatment. Slightly over a thousand cultures of the flies ("F_1") whose parents had been treated with varying doses of x-rays (called *t*1, *t*2, etc.) were started simultaneously, and nearly the same number of controls.

When the offspring ("F_2") from the above cultures began to be examined (November, 1926) a decided difference between the control and treated series was soon evident. The controls showed a very low frequency of lethal mutations (1 in 947 fertile cultures), like that usually found, whereas the treated series showed a surprisingly high frequency of lethals (88 in 758 cultures), and all but 3 of these lethals were confined to the chromosomes derived from the treated progenitor. They occurred in abundance, moreover, no matter whether the treated progenitor had been a female fly or a male. Similar results were found with regard to visible mutations and semi-lethals in these cultures, though of course they were not as numerous as the lethals. In later generations ("F_3" and "F_4"), only a small number of new mutations were found, not significantly more in the treated than in the control series.

A new series of crosses was then initiated (spring of 1927), similar in principle to the preceding, but in which the X-chromosome introduced from the untreated female progenitor contained the grouping of genes called "C_l," which prevents crossing over, and which is lethal in its effect on males. If now the offspring females, which were subjected to the breeding test, had also received a new lethal, in the X-chromosome from their treated father, they could not produce sons containing this X-chromosome, or sons containing the C_l chromosome either, that is, they could produce no sons at all—a condition definitely determinable by naked-eye inspection of their culture bottles. Somewhat over 1600 (fertile) cultures were examined in this set of experiments, and the results were substantially the same as before. In both sets of experiments, moreover, the treated progenitors were bred through two broods, and the offspring from the second brood, from germ-cells effecting fertilization a week or more after treatment, were found to have sensibly the same frequency of mutant genes as the others. On adding together the results of both sets of experiments, the lethal mutation frequency was found to be of the order of 150 times higher in the heavily treated *t*4 series than in the controls, and also significantly higher in the *t*4 than in the half as heavily treated *t*2 lots—something of the magnitude of one and a half times to twice as high, though the precise factor here is as yet uncertain.

To obtain more abundant data on visible mutations, rather than the ubiquitous lethals, crosses were made at the same time of treated males to females having attached X-chromosomes and a Y. Here the sons carry their mother's Y and their father's X and hence reveal on inspection all visible mutations, even recessives, that arose in this X of the treated sperm. Counts showed that the abnormalities thus produced were very abundant, and similar results have been obtained later on repetitions of this experiment.

Combining all the work done in 1926 and 1927, the following sex-linked

mutations had been induced (one or more times) that appeared allelomorphic or identical with previously known mutations: broad, white, eosin, facet, Notch, crossveinless, tan, vermilion, tiny bristles, miniature, garnet or ruby, small wing, rudimentary, uneven eye, forked, small eye, cleftoid and bobbed. Of these, at least one each of the following had been proved, by crossings with the old mutants, to be really allelomorphic to them: white, Notch, tan, vermilion, small wing, small eye, forked and bobbed.

The data on induced mutations in the X were sufficient to allow a graph to be made, showing their relative frequencies throughout the length of the "map." The graph disclosed that they were heaped more abundantly in the same regions as those in which more spontaneous mutations had previously been found. This unevenness is probably due merely to variations in our measure of length—the cross-over frequency—from region to region of the chromosomes.

Though most of the studies to date have been directed especially at the X-chromosome, various autosomal mutations also have been found, both dominants and recessives. In one experiment they were looked for at six specific loci (in a cross of x-rayed wild-type males by females homozygous for roughoid, hairy, scarlet, pink, spineless, ebony) and found at two of them (scarlet and spineless), though in each case combined with lethal effects that might suggest "deficiencies." Among the dominant autosomal mutations which have been induced are star eye, hairless, mutations resembling in appearance delta and truncate, and many of the minute bristle type; also dominant mutant allelomorphs of vestigial, and of eyeless (see below). The data on dominants indicate that mutations are induced as readily in the autosomal chromatin as in that of the X-chromosome.

The above lists include only those induced mutations which seemed the same as, or allelomorphic to, mutations already familiar to the Drosophila worker. There were also diverse new types of mutations produced, both in the X and in the other chromosomes, but space forbids their being passed in review here.

2. *Possible Nature of the Effect and Structure of the Gene.*—A considerable number of the induced mutations have been carried through a protracted series of breedings, and in the great majority of cases—with one exception, to be noted later—they breed quite true, as stable Mendelian genes. Their stability is in fact evident almost from the moment of their origination, for if the mutant gene mutated again, backwards or forwards, in the cells of the early embryo derived from the mutant germ cell, the resulting adult would show an irregular patchwork of mutant tissues, scattered among normal tissues. This is not the case. It can be shown however that, on the theory that the gene is compound, consisting of a

number of interchangeable molecules or larger members, this should probably be the case, that is, a patchwork of tissues should result, as mutant and non-mutant molecules became sorted out irregularly in the cell-divisions directly following the original mutation, and an irregularly patterned mosaic adult would be formed. We may conclude that it is likely that the gene ordinarily consists of not more than one molecule, or at least not of several molecules of the same kind.

But although the adult individual arising from a mutant germ cell does not show mutant and normal tissues mixed up in any such a crazy-quilt pattern, it does, usually at any rate, contain both mutant and normal tissues, and each of these tissues usually forms, so far as can be determined, one coherent sector. The germ cells may or may not be included in the mutant sector. This fractional character of the mutants seems most plausibly interpreted on the assumption that the chromosome, or at least the individual gene, in the parental treated sperm cell was already divided into two halves, and that only one half became transmuted.[3] If this is true the sperm cell in a sense contains twice as many genes as we thought it to, and our maximum estimates of gene size must correspondingly be cut in two, bringing them even nearer to the size of a single protein molecule.

That the transmuting action of the x-rays is thus spatially narrowly circumscribed, being confined to one gene even when there are two identical genes close together, has also been shown in another way. Females were treated (fall of 1927) which contained one X-chromosome having C_l, also a normally patterned X-chromosome, and a supernumerary Y-chromosome. When this combination occurs, many offspring are formed in which, owing to non-disjunction, both X-chromosomes from the mother are present at once. It was found, on analysis of 84 such "non-disjunctional" offspring by the breeding test, that in four of them a gene in one of these treated X's had mutated, without the corresponding gene in the other treated X having been affected. Two of these mutations were lethals and two were visibles. No case of a simultaneous mutation of two corresponding genes has been found. Hence, the accidental position of the gene in the path of the rays, rather than its chemical composition *per se*, may be of major importance in determining whether it happens to become transmuted.

More direct evidence for this conclusion was found in two cases in which it could be shown that two or more near-by loci, containing genes of different character, had become changed simultaneously. The more striking of these cases is that of notched wing and mottled eye, which arose together and which lie only about one and a half units apart in the chromosome; a breakage in the same chromosome, at a point not far removed from these, also occurred at the same time. This "group muta-

tion," or simultaneous occurrence of gene changes, in this case close together in a row, suggests that the secondary or β radiation, the released electrons, may be the effective agent, and the chance position of the gene in relation to the course of the electron may be a deciding condition in the production of the mutation. That the mutations are thus caused by chance absorption of individual quanta, such as may take place in the line of these secondary rays, is further suggested by the lack of relation between the amount of the x-ray dose and the intensity or the character of the mutations produced: their number varies with the x-ray dosage, but it seems that the "degree" or nature of the individual mutations themselves does not vary with the dosage.

Intensive study of the individual mutations is however called for if we would know more of the properties of the gene. Of the individual mutations, the mottled above referred to has been the most interesting and unique, it being the only example, among our induced mutations, of an "ever-sporting gene." Weinstein has recently reported another case. The continual mutations of mottled backward and forward, even in the somatic tissue, are indicated by the splotches on the eye, and different strains of it, having more and fewer spottings, and varying intensities and extensions of color in the spottings or in the background, have been established by selection. The theory of a compound gene might at first sight seem to find a very favorable case here, but it must be recalled that other allelomorphs of this gene are known (eosin, tinge, etc.) which, although they too show a color intermediate between red and white, are not unstable at all. Thus the instability of mottled would seem to depend on some other feature of the gene structure than the apparent mixture of color elements. Moreover, the variations of mottled are to some extent correlated with variations in the notching of the wings, though the notch gene is not identical with, or even directly adjacent to the mottled gene.

There are other individual mutations which can be used for intensive study, because their frequency of occurrence after raying is so high. For example, ordinary white eye (of which mottled is an allelomorph) has been produced something like twenty times, its frequency of occurrence, after the heavy treatment, being of the order of once in every thousand germ cells. The dominant, star eye, has been found to have the same or an even higher frequency. Rudimentary wing has occurred several times, so have miniature wing and various other types.

3. *Evidence for Other Alterations than Losses.*—In view of this ready repetition of given changes, by an agent known to be so often destructive in its effect, Altenburg has suggested a question which must be faced. Are the x-rays merely punching holes in the chromosomes, or, more precisely stated, simply causing losses or inactivations of genes or gene parts? If so, the usefulness of the x-ray, both from a practical and from a theo-

retical standpoint in biology, would be seriously limited. We have therefore attempted this year (1927–1928) to discover, at the suggestion of my wife, whether mutations could be produced in both of two opposite directions by x-rays—as from red to white eye and also from white to (or toward) red. In the case of this particular gene, extensive counts by Hanson, by Patterson and by myself have so far failed to show a mutation from white to (or toward) red, though such a change would have been found had it tended to occur with as high a frequency as the red to white mutation. Hanson has found four reverse mutations of the dominant bar eye to normal round eyes, but this change would, on the basis of Sturtevant's work, be a loss, and the opposite change, round to bar, has not yet been produced. Again, in the experiment previously cited which proved that only one of two corresponding genes was transmuted at a time, females were used which were homozygous for the mutant gene scute (in which bristles are missing on the scutellum) and the two visible mutations found in this experiment both consisted of reverse mutations of this "scute" to the dominant normal gene which causes the production of scutellar bristles. This occurred once in the C_l-bearing X-chromosome and once in the other X. Unfortunately, the opposite change—normal to scute—has not yet been observed in x-rayed material, although it has occurred several times in the past in other work (as has also the scute to normal change). This case, too, then fails to quite meet the conditions for the solution of our problem.

Recently, however, unexpected evidence from another angle, bearing on the point at issue, has been obtained. In the x-ray work this year, an "eyeless" (reduced eye) mutation of appearance similar to the familiar one, and also lying in the tiny chromosomes of the fourth pair, has been discovered, but this x-ray eyeless, unlike the old eyeless, is a *dominant*, that is, it shows as "eyeless" even if one normal fourth chromosome is present in the fly. Now, previous work of Bridges has shown that if one normal chromosome of the fourth pair is present, and if the other fourth chromosome has been completely lost, the flies (called "halpo-IV's") do not show the characteristics of eyeless (being normal eyed in these respects). The new dominant eyeless, therefore, does not seem to be due to a loss, since the real loss, *per se*, is known to give no dominant eyeless effect. Nor is the new eyeless a mere "duplication," for an extra fourth chromosome, in Bridges' experiments, produced no such eyeless effect either. The dominant eyeless accordingly appears to consist of some other kind of alteration in the gene than a change purely quantitative in its nature. There is only one objection of any importance to this conclusion, though perhaps it is rather far-fetched: that is, that the loss of a part of one chromosome might perhaps produce a different and, in some respects, a stronger effect than the loss of the whole chromosome would,

on the assumption that adjacent genes (or genetic materials) somehow interact in a special way by reason of their proximity.

A stronger case, not open to any of the above objections, has even more recently been worked out (April-May, 1928).[4] Here a mutation has been found, after raying, in which the gene for forked bristles reverted to the dominant normal bristles, and true-breeding stock of the reversed gene has been established. "Contamination" can be ruled out as an explanation here, since a peculiar chromosome inversion which had been present served as a check on this. It will be recalled that mutation in the direction, normal bristles to forked, had already been observed (several times) after raying. As the forked to normal change has never been observed without raying, and as the present reverse mutation was accompanied by another visible mutation, in a different locus of the same chromosome, it is highly unlikely that it was of "spontaneous" origin (i.e., not caused by the raying). Hence this case affords convincing evidence that changes other than losses can be produced by x-rays. It will be desirable, however, to search for further such cases of induced mutations in opposite directions, in order that quantitative data may be obtained concerning the potentialities of the genes.

4. *The Reorganization of Chromosomes.*—In the earlier experiments, besides the gene mutations, numerous cases were found that seemed to be so-called "chromosome mutations," or, more properly speaking, chromosome reorganizations, as they involved rearrangements of blocks of genes comprising whole sections of chromosomes. Usually, these "mutants" showed modifications in their cross-over frequencies, such as are taken to indicate that the chromosome has been broken, and a section of it turned around in its place or otherwise reattached abnormally to the remaining section, but some of the cases clearly involved an attachment between the broken-off fragment and some other, non-homologous chromosome. In order to obtain more definite data on the latter cases, which we call "translocations"—only one example of which seems to have been definitely established in previous Drosophila work, by Bridges in 1918, though Blakeslee in 1926 reported a case found, *after raying*, in Datura—Altenburg and I have this year (1927–1928) made certain crosses of treated flies to others having dominant genes, as markers, in two of their main autosomes, and the offspring have been bred to discover such infractions of Mendel's second law, the law of "random assortment," as would result from an attachment between pieces of non-homologous chromosomes. We find that such translocations, after x-ray treatment, are nearly as abundant as are the (detectable) gene-mutations. More than 70 have so far been found, including attachments between one long autosome and another, between either long autosome and the X, and between either long autosome and the Y, and also two cases of double translocations

involving all three long chromosomes simultaneously, in such a way that the animals bred as if they had only one pair of very long chromosomes (like *Ascaris univalens*). This is the number of such double events to be expected if the different translocations really occur independently of one another. Analysis of all the data shows that there is little or no preference for attachment between particular chromosomes, it being a chance affair, except that the likelihood of a given chromosome being broken, or of its serving for the attachment of the fragment of another, varies approximately with its own length.

Breakages, and also attachments, may occur at various points along the chromosome "map," that can in many cases be determined by genetic evidence. We may, then, examine the cells under the microscope, and verify our picture as genetically drawn. This problem has now been attacked, and figures have been obtained by the writer and by Altenburg, in several cases of translocation, which seem plainly to meet the genetic expectations. However, Drosophila cytology is elusive in its finer details, and so it will be desirable to obtain a fair number of incontrovertible and mutually confirmatory figures of each case before the conformation in question can be considered as conclusively established. Professor T. S. Painter is now working intensively on a number of cases of this sort. It should be noted that such cytological verification not only establishes the claims made with respect to the individual translocations involved, but also serves to prove directly that our genetic methods of reasoning are sound: that is, that the genes really do lie in the chromosome in linear arrangement, in the physical order in which we have theoretically mapped them—a cardinal principle which not all those who believe in the chromosome theory in a more general way have hitherto admitted. And with this proof, it can be shown, goes the proof of the corollary proposition, that the so-called "mechanical theory" of crossing-over is correct.

When the individuals containing translocations are bred, various points concerning the behavior of the chromosomes come to light. It is found, for example, that the translocated piece sometimes crosses-over with its normal homologue that is differently placed; this illustrates the fact that synapsis and crossing-over depend on an attraction between like genes rather than between chromosomes as a whole. Usually, however, cross-over *frequencies* are much affected in one way or another.

Among the zygotes formed on breeding individuals containing translocations, there are always two classes of "unbalanced" types, one of which receives a chromosome deficient in a section of its genes, but fails to get the translocated section that originally belonged there, and the other of which, conversely, receives an entire normal chromosome of this category, and gets the translocated section in addition. Often the individuals of such "unbalanced" compositions die, but occasionally they live, and then

they commonly show abnormalities caused by the unusual gene proportions; thus the somatic and genetic effects of missing or extra genes may be studied. Since these abnormal or lethal individuals are regularly produced when individuals with translocated chromosomes are crossed to normals, it is evident that the existence of such translocations in homozygous condition in a sector of a population will tend to isolate it physiologically from the rest, and so might become a factor in allowing divergence in evolution. The obstacles in the way of crossing-over and of free recombination in the case of translocations (and inversions) tends in the same direction.

5. *Generality of the Effect.*—Various other workers, preëminently Weinstein, have now confirmed the effectiveness of X-rays in producing both gene mutation and chromosome reorganization in Drosophila. In our laboratory Hanson, Patterson, Oliver and Harris have obtained definitely positive results. Recently Whiting, working on the wasp Hadrobracon, has extended the principle of gene change by X-rays to another animal than Drosophila. Stadler, working on barley and maize, has conclusively demonstrated, by means of ingenious methods, both gene mutations and chromosome aberrations ("non-disjunction?") to be produced in plants by x-rays. It should be stated, in this connection, that his work was carried on simultaneously with and independently of that of the present writer. The work of Goodspeed and Olson on tobacco, in which numerous phenotypic and also chromosomal abnormalities were clearly induced in the progeny of rayed flowers,[5] now extends the principle of heritable chromosome reorganization by x-rays to plants, and there seems to be a good opportunity for the production of gene mutations by x-rays also to be proved soon in this form.

The effect of x-rays on genes seems quite general in another sense as well. That is, the production of gene mutations by this means is not confined to a particular type of cell. In Drosophila, it is not only mature germ cells of both sexes which are susceptible, but also immature cells. For, in the earlier experiments (1926), it will be recalled that mutations were found not only in individuals from eggs laid immediately after treatment, but also in those from eggs laid a week or more afterwards, which must have been in the oogonial stage when the female was treated. In one such case, moreover, two daughters of the same treated mother were found to have an identical mutation (the semi-lethal, semi-dominant "cloven thorax," located near scute); these had obviously been derived from the same treated oogonium. Professor J. T. Patterson, now working on the general problem of effects on immature cells, finds that cells of the larva and early embryo may have their genes transmuted, and chromosomes eliminated or broken, by treatment given in the early stages: thus mosaic adults are produced bearing small or large patches of visibly

mutant tissue, each patch derived from one mutant larval somatic cell, while similar phenomena in the germ cells result in groups of identically mutant offspring. Stadler has reported analogous findings of groups of identically mutant offspring from barley rayed in the seedling stage. These genetic effects on "formative tissue" may also furnish the explanation for the effects of raying on the hair color of homozygous and heterozygous mice, reported by Hance.

6. *Is the Effect Unique?*—Before closing, we may raise the question: to what extent are x-rays unique in their effects? May not other maltreatment also produce mutations? It is to be expected that radium radiation, because of the physical similarity of its effects to those of x-rays, would produce similar results in this respect also, and results obtained this spring by Hanson (using the C_l method) are clearly positive on this point. In one of his experiments the gamma rays alone were allowed to reach the flies, and in these, too, mutations were produced in abundance. Proceeding down the spectrum in the other direction, Altenburg, in the spring of 1927, tried maximum toleration doses of ultraviolet rays from a mercury arc, on an extensive scale, and the results here were definitely negative, in the sense that there could have been no effect of nearly the same order as is produced by maximum toleration doses of x-rays. Of course, individual wave-lengths still deserve trial separately.

The writer has tried five different treatments this spring (1928), in addition to controls, using approximately 500 cultures of the C_l type in each case, and examining them primarily for lethals. The controls yielded 2 lethals. One treatment consisted of the inclusion of one per cent of lead acetate in the food, on which the flies were placed from the egg stage through maturity; another treatment involved a similar protracted feeding on arsenic trioxide, in a semi-lethal concentration (0.015% of the food); in a third treatment, semi-lethal doses of manganese chloride (0.62% and 0.31% of the food) were administered in like manner. In all of these cases combined there was only one lethal (and no other) mutation observed (the lethal was in a manganese culture), and we may assume with confidence that, if the frequency of lethal mutations was low here, the general frequency of visible mutations must have been much lower still. This result, therefore, is at variance with that reported for butterflies by J. W. H. Harrison, since this author concludes that *visible* mutations are produced in abundance, both by concentrations of lead and of manganese far weaker than those here employed. Of course, it may be held that flies and butterflies would be affected totally differently.

The other two treatments tried on the flies consisted of semi-lethal doses of the vital stain, Janus green (0.25% of the food), and semi-lethal, partially sterilizing exposures to a temperature of 36°C. for from 40 to 64 hours. In the former experiments 2 lethals and 3 semi-lethals, and

in the latter experiment 4 lethal mutations were obtained. These numbers are not significantly higher, from a statistical standpoint, than the figure of 2 lethals gotten in the controls, yet there may have been a comparatively slight effect here, and a repetition of these treatments will be desirable. The apparent effect, however, is in neither case larger than that already known to be produced by moderately high temperature when this is applied for a week or more to non-radiated material (experiments of Altenburg and of the author, 1919–1926), and such an influence of temperature may conceivably only consist in rendering more effective that natural radiation which commonly penetrates even "non-radiated" cells (just as Holthusen, followed by Dognon, has found, in Ascaris eggs, that a rise in temperature may result in a marked increase in the injurious effect on development, of x-rays artificially applied). It may be repeated, however that if there was any effect in either of these two experiments, it was too small to be really demonstrable by the technique employed, and this supposititious effect must have been of an intensity at least three "octaves" lower than that of the x-ray effect.

Thus we are brought before the question: are all mutations ultimately due to rays of short wave-length and to high-speed particles of corresponding energy content? *If* so, biological evolution has been made possible only by the stray radiation present in nature—the beta and gamma rays, and the cosmic rays. This question permits a definite solution, for some organisms at any rate, if only we can compare the mutation rate in ordinary controls with that in cultures from which a large part of the natural radiation has been artificially excluded, but great numbers of cultures will be necessary for a study involving only such low mutation rates. Moreover, absolutely all radiation cannot be excluded, for, as J. B. S. Haldane has pointed out in this connection, there would always be some "residual radiation" associated with the necessary potassium of living things. In this projected experiment the coöperation of physicists will be desirable in the working out of effective means for securing the physical conditions required.

Altenburg, E., 1928. "The Limits of Radiation Frequency Effective in Producing Mutations," *Amer. Nat.*, Sept., **1928.**

Belling, J., and A. F. Blakeslee, 1926. "On the Attachment of Non-Homologous Chromosomes at the Reduction Division in Certain Chromosomes of Daturas," *Proc. Nat. Acad. Sci.*, **12**, 7–11.

Blakeslee, A. F., 1926. Address in *Proc. Internat. Cong. Plant Sci.* (in press).

——. 1927. "The Chromosomal Constitution of Nubbin, a Compound ($2n + 1$) Type in Datura," *Proc. Nat. Acad. Sci.*, **13**, 79–85.

Bridges, C. B., 1921. "Genetical and Cytological Proof of Non-Disjunction of the Fourth Chromosome of Drosophila Melanogaster," *Proc. Nat. Acad. Sci.*, **7**, 186–92.

——. 1923. "The Translocation of a Section of Chromosome II upon Chromosome III in Drosophila" (Abstr.), *Anat. Rec.*, **24**, 426.

Dognon, A., 1925. "Coefficient de temperature de l'action des rayons X sur l'oeuf d'Ascaris," *Compt. Rend. Soc. Biol. Par.*, **92**, 1389–92.

Gager, C. S., and A. F. Blakeslee, 1927. "Chromosome and Gene Mutations in Datura Following Exposure to Radium Rays," *Proc. Nat. Acad. Sci.*, **13**, 75–9.

Goodspeed, T. H., and A. R. Olson, 1928. "The Production of Variation in Nicotiana Species by X-Ray Treatment of Sex Cells," *Proc. Nat. Acad. Sci.*, **14**, 66–69.

Hance, R. T., 1928*a*. "The Reaction of Homozygous and Heterozygous Adult Characters under the Influence of X-Rays" (Abstr.), *Anat. Rec.*, **37**, 172.

——. 1928*b*. "Altering a Matured Genetic Character," *Jour. Hered.*, **18**, 377–80.

Hanson, F. B., 1928*a*. "The Effects of X-Rays on Productivity and the Sex Ratio in Drosophila Melanogaster," *Amer. Nat.*, **62**, 352–362.

——. 1928*b*. "The Effect of X-Rays in Producing Return Gene Mutations," *Science*, **67**, 562–3.

Hanson, F. B., and F. M. Heys, 1928. "The Effects of Radium in Producing Lethal Mutations in Drosophila," *Science*, **68**, 115–116.

Harrison, J. W. H., and F. C. Garrett, 1926. "The Induction of Melanism in the Lepidoptera and Its Subsequent Inheritance," *Proc. Royal Soc.*, **99B**, 241–63.

Holthusen, H., 1920. "Beitrage zur Biologie der Strahlenwirkung," *Arch. ges. Physiol.*, **187**, 1–24.

Mohr, O. L., 1919. "Mikroskopische Untersuchungen zu Experimenten über den Einfluss der Radiumstrahlen und der Kältewirkung auf die Chromatinreifung und das Heterochromosom bei Decticus verruccivorus," *Arch. mikr. Anat. Entw.*, **92**, Abt. II, 300–68.

Muller, H. J., 1926. "The Gene as the Basis of Life," *Proc. Internat. Cong. Plant Sci.* (in press).

——. 1927*a*. "Artificial Transmutation of the Gene," *Science*, **66**, 84–87.

——. 1927*b*. "The Problem of Genic Modification," *Zeitschr. ind. Abst. Vererb.*, Sup-Bd., **1**, 234–60.

——. 1928*a* and *b*. "The Effects of X-Radiation on Genes and Chromosomes," abstracts in *Anat. Rec.*, **37**, 174, and *Science*, **67**, 82.

——. 1928*c*. "The Measurement of Gene Mutation Rate in Drosophila, Its High Variability, and Its Dependence upon Temperature," *Genetics*, **13**, 279–357.

Muller, H. J., and E. Altenburg, 1919. "The Rate of Change of Hereditary Factors in Drosophila," *Proc. Soc. Exper. Biol. Med.*, **17**, 10–14.

Patterson, J. T., 1928. "The Effects of X-Rays in Producing Mutations in the Somatic Cells of Drosophila Melanogaster," *Science*, **68**, 41–43.

Stadler, L. J., 1928*a*. "Genetic Effects of X-Rays in Maize and Barley," address to Genetics Section, A. A. A. S., Dec., 1927; title in *Anat. Rec.*, **37**, 176.

——. 1928*b*. "Genetic Effects of X-Rays in Maize," *Proc. Nat. Acad. Sci.*, **14**, 69–75.

——. 1928*c*. "Mutations in Barley Induced by X-Rays and Radium," *Science*, **68**, 186.

Stein, E., 1922. "Uber den Einfluss der Radiumbestrahlung auf Antirrhinum," *Zeitschr. ind. Abst. Vererb.*, **29**, 1–15.

——. 1926. "Untersuchungen uber die Radiomorphosen von Antirrhinum," *Ibid.*, **43**, 1–80.

Weinstein, A., 1928. "The Production of Mutations and Rearrangements of Genes by X-Rays," *Science*, **67**, 376–7.

Whiting, P. W., 1928. "The Production of Mutation by X-Rays in Hadrobracon," *Science*, **68**, 58.

[1] This paper includes, besides new material, most of that presented by the author at the A. A. A. S. meeting, Dec., 1927.

[2] Department of Zoölogy, Contribution No. 221.

[3] Since the above was written Dr. Robertson has shown me figures (unpublished) in which a longitudinally split condition of the chromosomes in the spermatids of grasshoppers is evident, and Dr. M. T. Harmon has informed me that she has observed the same phenomenon in the spermatids of guinea pigs. Various cytologists have observed a split condition of chromosomes in the telophase stages of other cell divisions. According to observations of Shiwago, chromosomes are double at all stages, and split secondarily just before the identical halves separate in mitosis.

[4] This paragraph has been inserted since the reading of the original paper, as the reversion was not observed until just after the author's return from the meeting. In the original paper it had been stated that no reversions of forked had been obtained. The writer wishes to thank Professor J. T. Patterson for invaluable aid in this experiment, rendered during the writer's absence on the trip.

[5] E. Stein (1922, 1926) had previously reported numerous phenotypic abnormalities of Antirrhinum, produced by radiation, which were inherited through vegetative reproduction. She also noted abnormalities of chromosome distribution occurring at the reduction division, long after treatment. In animals, non-disjunction of the X and other chromosomes at maturation, produced by radiation, was first demonstrated by Mohr (1919) in *Decticus*.

ON THE ROOTS OF THE DERIVATIVE OF A POLYNOMIAL

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Communicated July 20, 1928

The purpose of this note is to indicate the nature of some results obtained by the writer in generalizing a group of theorems proved several years ago by Professor Walsh of Harvard University concerning the approximate geometric location of the roots of the derivative of a polynomial.[2] A principal result is the following

Theorem. Suppose m_i roots of a polynomial $f(z)$ of degree n have as their common locus a region C_i consisting of the interior and the circumference of the circle

$$C_i = x^2 + y^2 - 2\alpha_i x - 2\beta_i y + \gamma_i = 0,$$

where $i = 1, 2, \ldots, q+1$, *and* $\sum_{i=1}^{q+1} m_i = n$.

Then the roots of the derivative of $f(z)$ have as their locus

(*a*) *the common points of any two of the regions C_i;*

(*b*) *the points of every region C_i for which $m_i \neq 1$; and*

(*c*) *the interior and the boundary of all the ovals of the q-circular 2q-ic curve*

$$\sum_{i=1}^{q+1} \frac{nm_i}{C_i} - \sum_{\substack{i=1 \\ j=i+1}}^{q+1} \frac{m_i m_j \sigma_{ij}}{C_i C_j} = 0, \qquad (1)$$