The Chemical Production of Mutations

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A GENETIC MUTATION IS A CHANGE, presumably chemical in nature, in one of the genes which compose the chromosome thread. The mutated gene is as stable as the original; it goes on reproducing replicas of its mutated self and thus initiates a new hereditary line. It is believed that without mutation life would never have proceeded beyond its very first elementary beginnings. Yet the mechanism of this important process is practically unknown. With the discovery by H. J. Muller (*12*) that mutations can be produced artifically by X-rays, a new approach to the problem of mutation was opened up, and many new facts relating to this problem were brought to light. One of the most important results was the discovery that the term "mutation" includes a number of distinct processes. In addition to gene mutations as defined above, X-rays produce breaks in the chromosome threads; when the resulting fragments join together in novel combinations, so-called chromosome rearrangements, hereditary changes closely resembling mutations may be produced. Rules connecting quantity and quality of radiation with type and frequency of mutations were discovered, and inferences could be drawn on number of ionizations required, size of the sensitive gene volume, etc. (*10*). Yet the actual processes of gene mutation and chromosome breakage—if indeed these are essentially different from one another—are still as mysterious as ever.

X-rays are destructive and nondiscriminating. It is conceivable that less severe methods of producing mutations might make possible a closer insight into the processes concerned. An advance in this direction was made when it was found that ultraviolet light, too, is capable of producing mutations. The restriction of the effective wave length to a comparatively narrow range which includes the absorption bands of nucleic acid and certain protein components supported the hypothesis, put forward by radiation geneticists, that the first step toward the production of a mutation consists in the absorption of an energy quantum by some constituent of the chromosome. But the hope that more specific effects, dependent on the wave length, might be produced, was not realized. Certain differences between the action of ultraviolet and that of the much shorter waves of X- and gamma rays are not yet understood, but may eventually prove helpful in the analysis of the mutation process (*18*).

Chemical substances with mutagenic properties should be particularly useful tools for attacking the problems of mutation. If, as we assume, a mutation is a chemical process, then knowledge of the reagents capable of initiating this process should throw light not only on the reaction itself, but also on the nature of the gene, the other partner in the reaction. Moreover, it could be hoped that among chemical mutagens there might be some with particular affinities for individual genes. Detection of such substances not only would be of high theoretical interest but would also open up the long-sought-for way to the production of directed mutations.

The search for chemical mutagens has been going on for well over 20 years. The choice of substances tried for the purpose was mainly random. Iodine, ammonia, metal compounds, and carcinogens are only some out of the great number tested. Results were often clearly negative, and no clearly positive ones had been obtained up to the beginning of our experiments in 1940 (see *1*, *9*, *19*). It is obvious that a chemical mutagen must possess very special properties. It must be able to act selectively on the genic material without at the same time destroying the cell which contains this material. It was, therefore, only to be expected that many substances would have to be tried before an effective one was found, and the search was continued by many workers. This search was encouraged by the accumulation of data which pointed to an influence of physiological conditions, such as age (*13*), sex (*1*), starvation (*15*), and of the genotype itself (*5*, *11*, *16*) on mutation rate. If, thus, chemical conditions created by the organism itself are capable of influencing the process of mutation, it did not seem beyond hope that chemical substances introduced from outside might have similar effects.

The choice of mustard gas for trials of this kind was suggested by observations pointing to its interference with cell division. Mustard gas burns, like X-rays burns, heal only with difficulty, and even after they appear to have healed they have a tendency to break down again. In addition, it was found that vaginal epithelium of an ovariectomized mouse which has been exposed to a weak solution of mustard gas fails to manifest the mitotic activity which normally follows stimulation with estrogens, and that this inhibition of mitosis lasts for several weeks after the exposure to mustard gas. It is well known that the chromosome breaks and rearrangements caused by X-radiation interfere with cell proliferation, partly through mechanical disturbances of mitosis and partly through death of those cells which, after distribution of the fragments and new chromosome

combinations into the daughter cells, do not receive a sufficiently normal set of chromosomes. It was thought possible that mustard gas, like X-rays, may inhibit cell division through direct action on the chromosomes.

In the autumn of 1940, experiments were started to find out whether mustard gas is capable of producing gene mutations and chromosome rearrangements. *Drosophila melanogaster* was used as test animal. The flies were exposed to mustard gas vapor, first in a closed chamber and later in a container through which air mixed with mustard gas was sucked. The first results immediately gave promise of success. Both males and females became sterilized to a degree which depended on the dose. Sterility was found to be due to two independent causes, both of which are also known to be involved in the production of X-ray sterility. First, gametogenesis is inhibited, so that after a time no more ova and spermatozoa are available. Second, lethality is high among zygotes from treated eggs and, more important still, among eggs laid by untreated females which have been mated to treated males. Since the spermatozoa do not lose their motility as a result of the treatment, the most likely explanation was that mustard gas, like X-rays, produces chromosome breaks and rearrangements in the sperm.

In order to obtain conclusive proof that mustard gas exerts an action on the chromosomes, genetic methods for the detection of mutations were applied. Male flies were mated to untreated females, and the progeny (F_2) was examined for the occurrence of mutations. Early mutation work, as well as some more recent work on organisms which are genetically less thoroughly known than *Drosophila*, has suffered from the impossibility of eliminating the large personal error, for a trained worker may spot abnormalities which may pass unnoticed by a less experienced or less observant person. In *Drosophila* genetics this obstacle has been removed by methods which, in the main, have been designed by H. J. Muller, and without which the quantitative analysis of genetic radiation effects would have been impossible. The essential feature of these methods is their restriction to the detection of so-called lethal mutations, *i.e* mutations which are so harmful that they prevent development of the individual. Hence, lethal mutations are detected by the absence from the progeny of a whole class of flies, and since presence or absence are characteristics about which any two observers are likely to agree, these methods reduce the personal error to a minimum, while at the same time allowing the study of large samples without excessive labor. Particularly useful for large-scale tests are methods like the famous *ClB* test which are designed to detect sex-linked lethals, *i.e.* lethals on the sex chromosome, of which the male has only one, while the female has two. A sex-linked lethal prevents the development of a male carrying it, while it usually does not interfere seriously with development of the female. In the *ClB* test each treated or control sex-chromosome becomes subsequently represented, in the F_2, by a whole culture of flies, and if a lethal has arisen on a sex-chromosome, the corresponding culture will consist entirely of females—a fact which is, of course, readily observed even by an untrained person.

The result of the first *ClB* test with mustard gas, carried out in April 1941, was spectacular beyond expectation. Whereas the rate at which sex-linked lethals arise spontaneously in laboratory stocks rarely approaches 1 per cent, 90 lethals were found in about 1,300 treated sex-chromosomes. This represents a mutation rate of over 7 per cent. Only 3 sex-linked lethals were found in an equivalent number of untreated chromosomes, representing a rate of 0.2 per cent. Similar results had previously been obtained only with X-rays or other high-energy radiation. Further tests fully confirmed and even exceeded the first success, up to 24 per cent lethals being produced. Higher percentages can hardly be expected because, concomitantly with the increase in mutation rate, sterility becomes more and more severe.

Genetic analysis of the lethals produced in the first *ClB* test indicated that some of them were due to, or combined with, chromosome rearrangements, and these findings were confirmed by cytological examination carried out by Dr. Slizynski. A special test for the production of chromosome rearrangements by mustard gas was undertaken in December 1941. The method was designed to spot translocations, *i.e* rearrangements through which two chromosomes have exchanged portions with one another. Spontaneous translocations are so exceedingly rare that the use of controls was not considered necessary. The result left no doubt about the capacity of mustard gas to produce chromosome rearrangements; 7 translocations were found in 816 treated nuclei. A report on these results was sent to the Ministry of Supply in March 1942, but, like all this work, could not be published because of the security ban on work with war gases. In subsequent experiments more translocations as well as other types of rearrangements were produced. Since only *Drosophila* had been used for all these studies, it was gratifying that cytological investigations on pollen mother cells of *Tradescantia*, carried out by Dr. Koller in 1943, fully confirmed our finding that mustard gas can produce chromosome breaks and rearrangements.

The similarity between the genetic effects of mustard gas and of X-rays are so striking that only gradually did certain differences between the two types of action come to light. Yet special interest attaches just to these differences, because a comparison between chemical and physical mutagens seems a hopeful approach to the problem of mutation. The first difference appeared in work on translocations. It has been shown that the frequencies of X-ray-induced lethals, on the one hand, and of X-ray-induced translocations, on the other, bear a mathematical relationship to the dose administered,

the first increasing directly as the dose, the second approximately as its 3/2th power (*10*). Consequently, for a given dose of X-rays (as measured in roentgen units) there exists a numerical relationship between the numbers of lethals and translocations produced. Thus, a dose of 3,000 r-units produces about 9 per cent sex-linked lethals and about 6 per cent translocations between chromosomes II and III of *D. melanogaster*. After mustard gas treatment, this relationship is shifted very markedly in favor of sex-linked lethals. Instead of the expected 6 per cent, only 0.5 per cent translocations between chromosomes II and III were produced in an experiment in which the rate of sex-linked lethals was 9 per cent, and a similar relative shortage of translocations was observed in subsequent tests. At first sight, these observations seem to indicate that mustard gas is less efficient than X-rays in breaking the chromosome thread. However, it is well to be cautious in drawing this conclusion. It has to be kept in mind that with the methods used we could not detect the primary breaks, but only a proportion of the subsequently formed rearrangements. It is conceivable that chemical treatment interferes with the process of rejoining of broken ends in such a way that a given number of breaks results in fewer observable rearrangements than would be formed by the same number of X-ray breaks. Special tests are required to decide this point.

On the other hand, mustard gas does not seem to be less efficient than X-rays in the production of very small, so-called "minute" rearrangements. Slizynski and Slizynska (*17*), in a cytological of sex-linked lethals produced by various agencies, have found that in about 20 per cent of cases the genetic change underlying the production of a lethal is a minute deficiency in the chromosome, and this frequency appears to be the same after X-rays, after ultraviolet radiation, and after mustard gas treatment. These findings emphasize the similarity, often pointed out by geneticists, between true gene mutations and minute chromosome rearrangements, and they do not contradict the possibility that so-called gene mutations may be nothing more than chromosome rearrangements of so minute a size that they elude detection by cytological methods. It will be of great interest to determine whether small deficiencies form an equally high proportion of lethals which have been produced by less potent chemical substances than mustard gas.

A second difference between the actions of X-rays and of mustard gas came to light in the course of a study of visible mutations after chemical treatment. In one respect this study was disappointing since the mutations observed were of the same types as those found after X-ray treatment, and there was no indication of any specific effects of the gas on individual genes. Mustard gas seems to act as indiscriminately as X-radiation. There is, however, a difference between these two agencies which involves not the types of mutation which they produce, but the way in which the mutations become manifest in the offspring of the treated flies. After X-ray treatment of males most of the mutated offspring show the induced abnormality (such as yellow body color instead of the normal gray) over the whole surface of their body. Only a small proportion (less than 15 per cent) of the mutated individuals are mosaics, *i.e.* show the abnormality in a part of their body, the remainder being normal. In the progeny of mustard-gas-treated males, on the other hand, mosaics form a high proportion (usually between 30 and 50 per cent) of all mutated individuals (*2*). Moreover, whereas the gonads of X-ray mosaics rarely contain both normal and mutated cells, those of mustard gas mosaics quite frequently appear to do so. A special study has been made of such "gonadic mosaicism" with respect to sex-linked lethal mutations (*3*). A female, daughter of a treated male, whose ovaries contain a patch of tissue in which the cells carry a sex-linked lethal, will have fewer sons than a normal female, the shortage of sons depending on the relative sizes of the normal and mutated portions of the ovary. A similar depression of the sex ratio occurs also in the progeny of females who carry, evenly distributed through all cells of their ovaries, a sex-linked "semilethal" mutation, *i.e.* a mutation which weakens the males so that only a proportion of them are capable of completing development. Analysis of females giving a low sex ratio has shown that among daughters of irradiated males this abnormality is almost always due to a semilethal mutation affecting the whole of the ovary. On the other hand, in 9 out of 20 daughters of mustard-gas-treated males, the shortage of sons was due to the presence in their otherwise normal ovaries of a mosaic patch carrying a sex-linked lethal. Finally, mention should also be made of a striking case of mosaicism in which a son of a mustard-gas-treated male was, both in the gonads and in the soma, a mosiac for two different mutations of the same gene, although it must be assumed that in the treated spermatozoon each treated gene was present only once.

An explanation which seems particularly satisfactory in accounting for all these observations is that the gene affected by treatment does not always mutate at once, but may acquire a tendency to mutate which remains latent until a later cell division. Support for this hypothesis was obtained when it was found in several cases that the offspring of gonadic mosaics for a mutation again were gonadic mosaics for the same mutation. In these cases an induced specific instability seems to have been transmitted from one generation to the next before giving rise to a stable change. No parallel observations have been reported in literature on radiation genetics; but it seems worth noting that so-called "unstable" genes, *i.e.* genes which tend to mutate repeatedly in the same direction, have been found several times in untreated material (*6*).

The difference between the mutations produced by short-wave radiation and chemical reaction may be tentatively ascribed to the different amounts of energy involved in the two types of reaction. In short-wave radiation the energy made available is usually sufficiently large to produce a catastrophic alteration in the structure of the gene, by transforming it from one stable configuration to another. On the other hand, reaction of the gene with a chemical substance, because of the smaller amount of energy involved, may produce a less drastic effect, by transforming it to an intermediate metastable configuration. Such a configuration will, of course, tend to undergo "spontaneous"alteration to another and more stable configuration, *i.e.* an "unstable" gene is produced.

After the first positive results with mustard gas had been obtained, the search for chemical mutagens was extended to substances which either in their chemical structure or in their pharmacological action are related to mustard gas. Mustard gas is a fixative of protoplasm with unusual power of penetration. It was soon seen that these two properties by themselves are not sufficient to make a substance mutagenic, for neither osmic acid nor picric acid affected the mutation rate in tests in which the majority of the treated individuals were killed. Neither can it be said that every potent vesicant is a mutagen, for lewisite gave entirely negative results in two *ClB* tests. So far only three substances have been found which give genetic effects similar to those of mustard gas. These all belong to the class of nitrogen- or sulphur-mustards. Their chemical formulas are: (1) $O(CH_2 \cdot CH_2 \cdot S \cdot CH_2 \cdot CH_2Cl)_2$, (2) $N(CH_2 \cdot CH_2Cl)_3$, and (3) $CH_3 \cdot N(CH_2 \cdot CH_2Cl)_2$.

As the chemical mutagens presumably attack the genic material directly, it was noted with interest that these active compounds all contain an unsaturated atom (:S or ⋮N) which might combine with materials composing the gene, and that this activity would be enhanced by the type of side chains present in the vesicant mustards (e.g. $ClCH_2 \cdot CH_2$-). With this type of structure is associated the tendency to intramolecular cyclization to form onium compounds (*7*), characteristic of the active ⋮N and :S vesicants. Assuming that the mutagenic action is due to the reaction on the unsaturated atom of the uncyclized compound with the gene, it was thought likely that replacement of the :S and ⋮N by :O would not produce active materials, as the addition compounds of divalent oxygen are not stable in aqueous solution (and "O mustard" is not a vesicant). Stable addition compounds are, however, formed by compounds having the :CO group, and though the corresponding "CO mustards" were not tried, the tear gases, chloracetone and dichloracetone (compounds with $ClCH_2$- side chains instead of the $ClCH_2 \cdot CH_2$- of mustard gas) were examined. The activity of these compounds was extremely weak; in fact, the results were not clearly positive, although they suggest the possibility that both these compounds are slightly mutagenic. By analogy with the vesicant action of the :S compounds, the chlorethylacetones might be more active. It has not yet been possible to test these substances.

Other groups which might replace the :S and ⋮N of the mustards and retain the additive ability of the molecule include ⋮As, although compounds with ⋮As are rather unstable under physiological conditions and are mostly toxic. The corresponding ⋮P compounds are apparently out of the question in this connection, as they are spontaneously inflammable in air. The $:SO_2$ compounds may similarly be expected to show some activity, but not the :SO ones. This parallels their vesicant action. There is also the possibility of using nitrile, isonitrile, and the corresponding thiocyanates and isothiocyanates for the coordinating group, and attention was therefore directed to allyl isothiocyanate. A weak but definite mutagenic activity could be demonstrated for this compound (*4*).

On the other hand, this type of chemical structure does not seem a necessary prerequisite for a mutagenic substance. This is shown by the fact that Hadorn and Niggli (*8*) have obtained considerable numbers of mutations by exposing explanted ovaries of *Drosophila* to weak solutions of phenol.

Allyl isothiocyanate, or mustard oil, occurs naturally in plants of the genus *Brassica*. We do not know whether it acts as a mutagen in these plants, but it is interesting to speculate how far naturally occurring mutagens may be responsible for spontaneous mutability. It has been shown by Muller and Mott-Smith (*14*) that cosmic radiation and natural radioactivity are quantitatively insufficient to account for the observed rates of spontaneous mutation. Timoféeff-Ressovsky, Zimmer, and Delbrück (*20*) have suggested that random temperature oscillations inside the nucleus may occasionally overstep the energy threshold required for the production of a mutation. In the light of the results reported here it seems possible, however, that a certain proportion of natural mutations may be due to the action of mutagenic substances within the organism, and whose production may itself be the consequence of gene action. Such an assumption finds support in the known cases in which definite genes influence mutability of the rest of the genes or even of a specific gene (*2*, *11*, *17*). Search for natural mutagens is therefore of high interest, but may well turn out to be exceedingly difficult. By its very nature a natural mutagen can have no drastic effect in the species in which it occurs; otherwise, the species could not survive. Moreover, a physiological system which includes mutagens whose production is controlled by genes will, in the course of its evolution, have attained a finely attuned equilibrium between the strength of the effective substances and the sensitivity of the gene or genes on which they act. Removed from its normal genotypical environment, a natural mutagen may produce quite different effects or none at all. Therefore, results

obtained with one organism may not be transferable to another, in contrast to results gained with such drastic agencies as X-rays and, presumably, mustard gas. Nevertheless, it is tempting to consider the possibility that one of the means by which evolution adapts mutability to environmental requirements is the achievement of a balance between the production of mutagens and sensitivity to them.

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Obituary

Henry Helm Clayton
1861–1946

With the passing of Henry Helm Clayton, on October 26, 1946, there ended a life of exceptional activity and eminence in meteorology, public service, and business.

Clayton was born on March 12, 1861, at Murfreesboro, Tennessee. Because of delicate health, early education was acquired in private schools and by study at home. It was during this period that his interest in meteorology developed.

Studies of local storms, beginning in 1878, were followed in 1882 by his first activity, aid in the organization of the newly formed Tennessee Weather Service, including analyses of reports and a gift of 30 rain gauges. In 1884–85 he was assistant at the Observatory of the University of Michigan and associate editor of the *American Meteorological Journal.* In February 1886, after three months at Harvard College Observatory, he joined the staff of Blue Hill Meteorological Observatory, founded by Abbott Lawrence Rotch in the preceding year, where he remained as assistant and meteorologist until 1909. During this period many important advances were initiated by him with the enthusiastic approval of Director Rotch. At first there was no assistant, and at various times, at his own expense, Clayton employed others to share his rapidly expanding program of research.

Clayton's studies of clouds, begun in 1886, yielded the first definite information concerning the circulation of the atmosphere over America and established the Clayton-Egnell law of the increase of velocity with height. The detailed observations during the period 1886–90 are the only hourly data of changes in form, height, and movement of clouds in the Western Hemisphere, and, with Clayton's discussion, undoubtedly stimulated the organization of the International Cloud Committee and the international series of measurements of heights and velocities of 1896. The colored pictures for the *Atlas of clouds,* issued in 1897 by the U. S. Hydrographic Office, were painted under Clayton's supervision. He was also consultant in the preparation of the first *International atlas* (1905).

Clayton's invitation to William A. Eddy to try his meteorological kites at Blue Hill led to the first use of kites to lift recording instruments, August 4, 1894, and to the adoption throughout the world of this method of sounding the atmosphere. Important results of Clayton's analyses of the accurate data obtained included the discovery of persistent, sharply defined stratifications in the lower atmosphere, and previously unsuspected, variable effects of mountains upon the surrounding atmosphere. Interest in this new method of research, later to be named "aerology," is indicated by his generosity in allowing free use in aerology, without royalty, of the form of Hargrave kite patented by him, which came into use at all aeronautical laboratories. Always envisaging improved techniques and advances in aerology, he encouraged Blue Hill to develop the first radio sonde, in 1935.

He described the solar eclipse as "a kind of laboratory experiment in which are eliminated practically all influences upon the atmosphere except that of a fall of temperature," and original studies led to his suggestion of the eclipse cyclone caused by the cooling of the air by the shadow.

Clayton's great interest in forecasting was first indicated by his paper, "A lately-discovered meteorological cycle," published in the *American Meteorological Journal* in August 1884. Later, at Blue Hill, his proof that forecasts made locally are better than those issued at a