

RECOVERY FROM RADIATION AND CHEMICALLY INDUCED PREMUTATIONAL DAMAGE

By

NEIL G. BRINK

Department of Zoology, University of Tasmania

ABSTRACT

The question of recovery from radiation and chemically induced premutational damage is discussed in relation to the nature of the primary genetic damage at the molecular level, and also in relation to factors which influence the recovery process.

INTRODUCTION

Following Muller's (1927) discovery that gene mutations could be produced artificially in *Drosophila* by X-irradiation, subsequent investigations showed that X-rays, other ionizing radiations, ultra-violet light, and certain chemical substances produced mutations in a wide variety of organisms. However, it is only in comparatively recent years that the processes involved in the induction and expression of artificially produced mutations have been studied in any detail. Already it has become evident that these processes are subject to modification, and as a result the induced mutation frequency can either be increased or decreased depending on the nature of the modifying treatment. Hypotheses, which have been put forward in recent years, relating to the physical structure and mode of replication of genes at the molecular level, have served to stimulate these investigations by placing the study of mutation on a molecular basis.

Investigations carried out with bacteriophage (Zinder, 1955) and studies on bacterial transformation (Hotchkiss, 1955) strongly suggest that deoxyribonucleic acid (DNA), which is an important constituent of the chromosomal material, carries a part, if not all the genetic information. In the light of the model proposed by Watson and Crick (1953) to account for the structure and mode of replication of DNA, and of recombination experiments in bacteriophage, Benzer (1957) has proposed that the gene consists of a finite section of DNA containing a large number of small units (sites), which are inseparable by recombination experiments, and which may actually correspond to a single nucleotide pair or a few nucleotide pairs. An induced change (mutation) at any one site alters the expression of a gene, but changes at different sites within the gene locus alter the expression of the gene in different ways. Thus the presence of several alleles at any one locus, may, at the molecular level of the gene, be represented by mutations at several different sites within that gene locus.

On the basis of this model, there are a number of possible ways in which mutation could occur, some representing changes in an existing gene molecule, while others might be related to the synthesis of DNA and the replication of the gene.

(a) *Changes in already existing genes.*

- (i) Loss of one or more nucleotide pairs.
- (ii) Duplication of one or more nucleotide pairs.
- (iii) Loss of a base pair with the subsequent incorporation of an alternative base pair into the DNA molecule, or loss of a single base from one DNA strand and its replacement by an alternative base—with the incorporation of its complementary base in the other strand during DNA replication.
- (iv) The chemical alteration of a base, with its resultant change in behaviour leading to the incorporation of a different base in the complementary strand during DNA replication.
- (v) Fracture of the sugar-base bonds of a complementary pair of nucleotides, followed by the rotation of the attached base pair before re-establishment of the bonds.
- (vi) Intragenic changes produced by the inversion of two or more nucleotide pairs following breakage of complementary phosphate-ester linkages in both strands—four breaks in all.

(b) *Changes which are dependent on DNA synthesis for their induction.*

- (i) The chemical alteration of nucleic acid precursors with their subsequent incorporation into the DNA (or ribonucleic acid (RNA)?) molecule.
- (ii) A "copying error" during DNA replication in which a segment of DNA formed on an adjacent template or modified template (modified RNA protein template?), is incorporated into the daughter strand.

In addition to point mutations, other types of genetic damage involving chromosome breakage, for example, translocations, inversions, deletions, duplications and position effects, are all produced by radiations, as well as by some of the chemical mutagens. An understanding of the basic structure of the chromosome is necessary, in order to facilitate the study of processes involved in chromosome breakage and restitution. Our knowledge of chromosome structure is still somewhat fragment-

ary, but it is apparent that, chemically, the chromosomes are made up of nucleic acids and proteins. Structurally they consist of bundles of fibrils, the number of which varies in different organisms, but it is believed to be always some multiple of two (Ris, 1957). The exact arrangement of the nucleic acids and protein within the fibrils is not yet known, but it is thought that there is a central core of nucleic acids surrounded by an outer "coat" of protein.

It is very likely that different mutagenic agents induce genetic changes of different types or at least the same changes in different relative frequencies, whilst the same mutagen may produce mutations of different types in different organisms. Also, it is likely that treatments which bring about recovery from one type of mutational damage, will be ineffective against other types. Consequently, investigations into the recovery from induced genetic damage will not only help determine what types of damage are repairable, and the mechanisms involved in this repair, but they will also help to elucidate the steps involved in the production of the various types of mutations, and determine whether any of these steps are the same for radiation and chemically induced mutations.

It should be emphasized that treatments which modify the mutation frequency may not necessarily do this through interference with the recovery mechanism, but may either increase or decrease the amount of primary damage.

I. RECOVERY FROM RADIATION INDUCED MUTATIONS

A. Bacteria

Recent investigations have shown that at least a portion of ultraviolet (UV) induced mutations require protein synthesis (Witkin, 1956) as well as RNA synthesis (Doudney and Haas, 1959) for mutation fixation, whilst the terminal process in the induction of mutation apparently involves DNA synthesis (Haas and Doudney, 1959, 1960).

Witkin, in her experiments with the tryptophane requiring LT2 strain of *Salmonella typhimurium*, showed that post-irradiation incubation with the antibiotic chloramphenicol, an inhibitor of protein synthesis, reduced the frequency of induced prototrophs, whilst the frequency of survivors remained relatively unchanged. Thus the reduction in mutation frequency could not have been due to selection. Subsequently Doudney and Haas, in their experiments with the tryptophane requiring WP2 strain of *Escherichia coli*, found, by "challenge" with chloramphenicol or the base analogue, 6-azauracil, at various times after irradiation, that a relationship exists between the frequency of mutations "fixed" and the amount of RNA synthesized. Mutation fixation is apparently complete when the amount of RNA in the culture has doubled. DNA synthesis then begins and this apparently is the final step in mutation induction, since following DNA synthesis mutations are no longer susceptible to photoreversal (Haas and Doudney, 1960). Mutation expression is apparently dependent on protein synthesis, for it can be prevented by treatment with chloramphenicol following the initiation

of post-irradiation DNA synthesis, although the synthesis of DNA itself is unaffected (Haas and Doudney, 1959).

Investigations carried out within the last two or three years (Doudney and Haas, 1960a, b), have confirmed Witkin's observation that when chloramphenicol is added to incubating cultures immediately after irradiation, a decrease in mutation frequency occurs. However, Doudney and Haas found that a treatment of twenty minutes was sufficient to obtain maximum reduction in mutation frequency. Further treatment merely delayed synthesis of protein and RNA in the culture and this in turn led to a delay in DNA synthesis, but there was no further reduction in the mutation frequency. As a result of this finding Doudney and Haas (1960b) have proposed that "mutation frequency decline" is favoured by conditions which inhibit protein synthesis. However, this synthesis is not correlated with the macromolecular synthesis within the culture which leads to mutation fixation. Furthermore, "mutation frequency decline" is apparently an enzymatically controlled process, dependent on cellular respiration, since dinitrophenol (DNP) has been found to block the "mutation frequency decline" process promoted by chloramphenicol treatment. If "mutation frequency decline" does not occur, then the pre-mutational damage induced by UV is stabilized within the cell (mutation stabilization), and this stabilization process is apparently dependent on a supply of amino acids and conditions which favour protein synthesis.

Since mutation fixation is apparently dependent on macromolecular synthesis within the culture, it is evident that RNA and protein may play a part in DNA replication. The exact nature of DNA replication is still not known. However, Delbruck and Stent (1957) have found evidence in bacteriophage that it may involve an intermediary substance which may be nucleoprotein. This finding, therefore, would tend to support the views of Doudney and Haas that DNA replication in bacteria involves an RNA-protein intermediate.

Kanazir and Errera (1956) have found that DNA synthesis is temporarily inhibited following UV exposure in *E. coli*. This temporary inhibition may be due to a blocking of normal DNA synthesis, whilst resumption of synthesis may be brought about by the substitution of an alternative mechanism. It may be that this alternative mechanism involves the necessity of an RNA-protein intermediate, whilst normal DNA synthesis in the undamaged cell is along the lines originally proposed by Watson and Crick (1953). However, this is only speculation, and the actual role of RNA and protein in the production of UV induced mutations in bacteria has still to be determined.

Recent investigations carried out by Witkin and Thiel (1960) have shown that some UV induced mutations are apparently stable, since they fail to respond to the post-irradiation treatments which modified the frequency of the UV induced mutations discussed above. Thus, whereas the frequency of mutation from *try*⁻ to *try*⁺ is modified by post-irradiation treatment with chloramphenicol, the mutations from streptomycin sensitivity (*str*⁻*s*) to streptomycin resistance (*str*⁻*r*), and from

streptomycin dependence (*str* — *d*) to streptomycin independence (*stri* — *i*) were not affected by this treatment. Furthermore, Kada, Doudney and Haas (1961) have shown that X-ray induced mutations in bacteria are of two types; those whose frequency can be modified by post-irradiation treatment and those which fail to respond to these treatments. Thus Kada and his co-workers found that following X-irradiation of the WP2 and 15 *thy met tyr* (a thymine, methionine and tyrosine requiring strain) strains of *E. coli*, approximately half the mutants at the *try* and *tyr* loci respectively were expressed phenotypically in the absence of DNA synthesis, the other half requiring DNA synthesis for induction. "Challenge" with chloramphenicol or 6-azauracil during DNA synthesis showed that both types of mutations require protein synthesis for mutation expression.

The existence of these two types of mutations after UV and X-irradiation suggests that the primary damage is induced at two different sites. Thus in the case of those mutations whose frequency is modified by post-irradiation treatment with chloramphenicol, the initial damage may be to the nucleic acid precursors. Haas and Doudney (1957) have shown that addition of cytidine and uridine to cultures prior to irradiation increases mutation frequency, whilst the addition of these same compounds after irradiation decreases mutation frequency. On the basis of this finding, they have suggested that the addition of the compounds after irradiation reduces mutation frequency because of the competition between the added compounds and UV modified nucleic acid precursors for incorporation into RNA during its post-irradiation synthesis. On the other hand, the increase in mutation frequency resulting from the addition of the compounds prior to irradiation is presumably due to UV modification of the added compounds and their subsequent incorporation into RNA. Those mutations which fail to respond to post-irradiation treatment may be produced by the direct action of the radiation on the genes (deletions or intragenic inversions in the DNA molecule?), such mutations being phenotypically expressed without the necessity of DNA synthesis.

B. Paramecium

Kimball, *et al.* (1957) have shown that in *Paramecium*, a portion of the X-ray induced pre-mutational damage does not become finally established as an irreversible mutation until about midway through the interdivision period. They have found that incubation in streptomycin following irradiation produces a decrease in the mutation frequency, a treatment of four hours being sufficient to produce maximum mutation reduction. However, it was found that if the treatment was given at successively later intervals following irradiation, its modifying influence on the mutation frequency was progressively reduced. Further work by Kimball and his associates (Kimball, *et al.*, 1959) with stationary phase *Paramecia* has shown that there is a progressive decrease in the mutation rate during post-irradiation starvation.

On the basis of these two findings, Kimball and his co-workers propose that up to a point midway through the interdivision interval (called the intermediate period by Kimball) the induced pre-

mutational damage undergoes spontaneous reversal and only that pre-mutational damage which remains at the end of this period becomes "fixed" as a permanent mutation. This "fixing" may be correlated with DNA replication. Kimball suggests that an increase in the time available for loss of pre-mutational damage is the main factor responsible for "mutation frequency decline". However, it is likely that "mutation frequency decline" in *Paramecium*, as in bacteria, is a metabolically controlled process dependent on respiratory energy, since the rate of loss during exponential growth of paramecia was found to be very much greater than the rate of loss in stationary phase paramecia. Thus it is more likely that reduction in mutation frequency is not a spontaneous process and consequently is not increased by the lengthening of the interdivision interval, but is an active metabolic process dependent on a source of energy, but quite independent of the length of the intermediate period.

C. Drosophila

Independent investigations carried out by Nordback and Auerbach (1957), Abrahamson and Telfer (1956), and Lüning (1958) have shown that fewer translocations are found in mature sperm used on the second day after irradiation of males than on the first day. The frequency of other genetic changes, for example, sex linked lethals or Y chromosome loss, also seem to be lowered by storage of mature sperm in males following irradiation.

The translocation results would suggest that some restitution of induced breaks occur when the sperm are stored in the irradiated males. However, Oster (1955) has found that following irradiation of pupae, breaks induced in mature sperm remain open until fertilization, although breaks induced in spermatids apparently undergo restitution prior to fertilization. It is probable, however, that the environmental conditions experienced by the mature sperm in the pupal testes and in the testes of the adult fly may differ in some respects. Consequently, the fact that no restitution occurs in the pupae does not necessarily mean that no restitution occurs in the adult. Although mature sperm are relatively anoxic in *Drosophila* males, the oxygen tension may be sufficient to allow some restitution of the induced breaks before fertilization.

Alternatively, we may suppose that the initial effect of the radiations is not the breakage of the chromosomes, but the alteration of the chromosomes in such a way that breaks may be produced if the post-irradiation conditions are favourable (Thoday, 1953). On the basis of this hypothesis, it is evident that conditions in the testes of male imagos are favourable for the repair of these "potential" breaks, whilst conditions in the pupal testes and also in the spermathecae and seminal receptacle of females are unfavourable for this repair, since it has been noted by many workers that no reduction in translocation frequency or other types of genetic damage occurs when irradiated spermatozoa are stored in the spermathecae of inseminated females. Perhaps the transfer of the mature sperm from male to female or conditions within the spermathecae result in the transforma-

tion of potential breaks into actual breaks. Assuming that there is recovery from potential breaks when irradiated spermatozoa are stored in the males, the question arises whether this recovery is a spontaneous process dependent on time or whether it is an energy requiring process. In the latter case the metabolic activity of mature sperm must be sufficient to enable recovery to occur.

The question also arises whether there is recovery from point mutations when mature sperm are stored in irradiated males. Lüning, Schuwert and Jonsson (1958) and Lüning and Söderström (1957) have shown that breakage followed by restitution in the original order does not appear to have any influence on the frequency of sex-linked lethals. Hence, the decline in frequency of sex linked lethals following storage, reported by Nordback and Auerbach (loc. cit.), may be due to a reduction in position effect lethals, since it is accompanied by a similar fall in translocation frequency and was of the same magnitude.

The effect of the presence of oxygen during irradiation on the production of genetic damage, and also on the recovery mechanism in mature spermatozoa has been the subject of extensive investigation in recent years. Lüning and Hannerz (1957) and Lüning and Söderström (1957) have suggested on the basis of their findings using dose fractionation, that the initial amount of primary damage is the same after irradiation in nitrogen as after irradiation in air. Thus they conclude that irradiation in air apparently affects the recovery mechanism in some way. Furthermore, Lüning and Henze (1957) have shown that for a given total dose of 6480 r, increasing the proportion of this dose given in air up to 1080 r increases the amount of damage produced, but that no further increase occurs if the dose given in air exceeds 1080 r. They regard this saturation phenomenon as due to a complete inactivation of the rejoining system produced by doses in excess of 1080 r in air. However, Sobels (1961) does not support this proposal, since he has found that post-irradiation treatment with cyanide inhibits recovery to the same extent, irrespective of whether the irradiation is given in nitrogen or oxygen.

The recent findings of Wolff and Lindsley (1960) corroborate the findings of Lüning and his associates, that dose saturation occurs when the irradiation is carried out in air, but in experiments in which doses were administered in pure oxygen, no such dose saturation was observed. To account for this apparent anomaly Wolff and Lindsley have suggested that there may be two oxygen sensitive systems; the rejoining system which is sensitive to low oxygen tensions, and breakage, which is relatively insensitive to low oxygen tensions but is affected by high oxygen tensions. This is similar to the model proposed to explain the oxygen effects in root tips of *Vicia faba* (see below, Wolff and Atwood, 1954). Although no experimental evidence exists to support this proposal, it would be reasonable to assume that the various cellular components affected by irradiation will suffer damage to variable degrees depending on physiological conditions during and immediately after irradiation, different components being affected to different extents by these conditions.

Sobels (1960, 1961) has shown that recovery in *Drosophila* is apparently dependent on cellular respiration, since he has found that post-irradiation treatments with cyanide or azide increase the frequency of mutations (in this case sex linked lethals) produced in the spermatid stage. That this increase in mutation frequency is not due to an increase in the number of position effect lethals resulting from chromosomal rearrangements, but is due to an increase in the number of point mutations and small deletions, is shown by the fact that after irradiation at low intensities, translocation frequency is increased by post-treatment with cyanide, but the frequency of sex linked lethals remains unaltered. Also post-treatment following irradiation at high intensities increases the frequency of sex linked lethals induced in a ring-X chromosome. Cyanide itself has been found to be nonmutagenic in *Drosophila* (Sobels, 1954), whilst the original proposal that cyanide blocked the catalase system, thus allowing the accumulation of radiation produced mutagenic peroxides (Sobels, 1957), has been made untenable by the finding that post-treatment with cyanide enhances mutation frequency to the same extent, irrespective of whether the irradiation is carried out in nitrogen or oxygen (Sobels, 1960).

The premutational damage produced in spermatozoa and spermatids by X-irradiation presumably alters the pre-existing gene, since no DNA replication occurs until the time of the first cleavage in the fertilized egg (Muller, *et al.*, 1961). The finding of Altenburg and Browning (1961) that no quarter mosaics are produced following irradiation of sperm and spermatids supports this view.

Altenburg and Browning have also shown that very few half mosaics are produced following irradiation. This suggests that the radiation affects both strands of the DNA molecule and not just a single strand. As mentioned earlier, changes involving both strands of the DNA molecule may be produced by duplication or deficiencies of one or more base pairs, intragenic inversions or by rotational substitution. It may be that restitution of the sugar-phosphate linkages or sugar-base bonds broken by X-irradiation, is delayed by post-treatment with cyanide or other respiratory inhibitors, thus allowing more time for rearrangement of the detached nucleotides or base pairs respectively.

Although mutations produced in spermatozoa and spermatids seem to involve changes in the already existing gene, it may be that mutations produced in younger germ cell stages may depend on DNA replication for their induction. Perhaps treatments which proved effective in modifying mutation frequency in bacteria (e.g., chloramphenicol) may be effective in modifying the mutation frequency in younger germ cell stages in *Drosophila*.

D. Plants

Thoday and Read (1947) showed that irradiation of *Vicia faba* root tips in the presence of oxygen produced more genetic damage than irradiation in anoxia. It became apparent from further investigations in *Vicia* (Wolff and Atwood, 1954) and *Tradescantia* (Wolff and Luippold, 1958) that oxygen affects both breakage and rejoining of the broken chromosomes. However, apparently only the time of rejoining, and not the amount of rejoining is

affected. Therefore the decrease in aberration yield when the irradiation is carried out under anoxic conditions is due to fewer breakages being produced and not to increased restitution, although restitution occurs more rapidly after irradiation in nitrogen compared with the rate of restitution after irradiation in oxygen. Wolff and Luippold (1955) have also found that agents which inhibit oxidative metabolism such as low temperature, cyanide, carbon monoxide, anaerobiosis and DNP, increase the length of time that breaks remain open. From this it is apparent that chromosome restitution is an energy requiring process and therefore dependent on cellular respiration and the formation of ATP. Small amounts of oxygen are apparently sufficient to permit oxidative metabolism and therefore facilitate chromosome restitution. The addition of exogenous ATP, or of $MnCl_2$, which stimulates ATP synthesis in the mitochondria (Ernster and Low, 1955), enhances the repair process (Wolff, 1960).

Recent investigations (Wolff, 1960) with *Vicia faba* have shown that protein synthesis is necessary for the restitution of radiation produced chromosome breaks, since application of chloramphenicol and other protein synthesis inhibitors, notably aureomycin prevents restitution of chromosome breaks. How protein synthesis promotes chromosome restitution is not known, but Wolff has suggested that it may either involve direct action such as the synthesis of a peptide link or indirect action resulting from the formation of an enzyme or enzymes which may be necessary to promote the restitution of breaks.

E. Mice

Recent work by Russel, Russel and Kelly (1958) suggests that recovery from premutational damage may not be confined entirely to "lower organisms", but may actually occur in the spermatogonia and oocytes of mice following chronic irradiation. They found that specific locus mutation rates were lower after chronic gamma-irradiation than after acute X-irradiation in the spermatogonia and oocytes, but not in the mature spermatozoa. It was found that this difference in the mutation rate was not due to qualitative differences in the nature of the radiations, nor was it due to cell selection. To explain these findings, Russel and his co-workers have suggested that following irradiation at low intensities, a part of the premutational damage is repaired before it becomes finally established, but after irradiation at high intensities there is apparently no repair, presumably because the repair mechanism is damaged by acute irradiation. The failure of the spermatozoa to show any intensity effect is assumed to be due to the relatively low metabolic activity of the mature sperm compared with the spermatogonia and oocytes.

The existence of an intensity effect in certain germ cell stages of the mouse is of interest because of the conflicting results obtained with high and low dose rates in *Drosophila* (Clark, 1960a). Should further experimentation show that this intensity effect in the spermatogonia and oocytes of the mouse is due to differential recovery following chronic and acute irradiation, then perhaps this recovery phenomenon will need to be taken into

account when determining the genetic as well as the somatic hazards of ionizing radiations in human beings.

II. RECOVERY FROM CHEMICALLY INDUCED MUTATIONS

In contrast to the extensive investigations that have been carried out on recovery from radiation induced premutational damage, very few investigations have been carried out in order to determine whether chemically induced premutational damage is also subject to modification, and if it is, whether it is due to recovery.

Clark (1958) has found that injection of either cyanide or azide in *Drosophila* males failed to increase the mutagenic activity of injected pyronin. However, Sobels (1956), and Sobels and Simons (1956) have reported that the formaldehyde induced mutation frequency in *Drosophila* males and females respectively is enhanced if formaldehyde injection is preceded by treatment with cyanide gas. It has been suggested that this enhancing effect of cyanide is due to a blocking of the catalase enzyme system, which allows the accumulation of formaldehyde produced organic peroxides, the presence of these peroxides resulting in an increase in mutation frequency. If this explanation is correct, then the enhancement of mutation produced by cyanide gas is not due to interference with a recovery mechanism, but is due rather to a mechanism which increases the amount of primary genetic damage by allowing mutagenic peroxides to accumulate as a result of the inhibition of catalase.

The main groups of chemical mutagens are reviewed by Röhrborn (1960). Investigations carried out in recent years have done much to determine how the alkylating agents (see, for example, Stacey, *et al.*, 1958, Bautz and Freese, 1960, Fahmy and Fahmy, 1961) and formaldehyde (Alderson, 1961) react with the genetic material.

Alexander and Stacey (1958) discuss the genetic changes produced by radiations and alkylating agents. Undoubtedly the end results are the same, that is, the production of chromosome breaks and mutations, but it is still doubtful whether the intermediate steps are the same. The polyfunctional alkylating agents are much more potent with regard to the production of chromosome breaks than the monofunctional agents. Both groups of compounds are capable of breaking the phosphate-sugar bonds, although the polyfunctional agents, by forming cross-linkages between the two complementary strands of the DNA molecule, will be more effective in producing breaks in both strands (Fahmy and Fahmy, 1961). However, the mere breakage of the "backbone" in both strands of DNA is not sufficient to produce a mutation. Since the molecules of chemical mutagens have to diffuse into the cell, it may be that their effects will be more localised in contrast to the random effects of radiations. Consequently, the reaction of two or three molecules with a small finite section of DNA may produce several breaks in close proximity to each other, thus permitting the inversion of small blocks of nucleotide pairs with the resultant production of mutations.

Both Auerbach (1946) and Altenburg and Browning (loc. cit.) have reported a high percentage of mosaicism following treatment of *Drosophila* with alkylating agents. Thus Altenburg and Browning found that following the injection of phenylalanine mustard, dimethyl myleran or 2, 5 bis-ethyleneiminoquinine, the percentage of fractionals detected varied from 30% to 67%, compared with about 7% for radiation induced mutations. Most of these fractionals appeared to be half mosaics, consequently alkylating agents may produce the major part of their effects in one or other of the DNA strands, but not in both. For example, Bautz and Freese (1960) have found that ethylation of the 7N position of guanine following treatment with ethylethane sulphionate resulted in the hydrolysis of the 9N sugar-base bond and the resultant loss of guanine. Subsequent substitution of adenine would lead to mutation. It was also found that methylation of guanine produced less hydrolysis of the 9N sugar-base bond and this may account for its weaker mutagenicity.

Perhaps a study of factors which modify chemical mutagenesis, and in particular influence recovery from premutational damage will help to clarify the nature of the initial damage produced by the alkylating agents and by other chemical mutagens and enable a comparison to be made between this damage and that produced by radiations. With this end in view, investigations have begun in this laboratory to test whether the frequency of mutations induced by the pyrrolizidine alkaloid heliotrine can be modified by those treatments which proved effective in modifying the radiation induced mutation frequency in *Drosophila*. Little is known at the molecular level about the nature of the mutagenic activity of the pyrrolizidine alkaloids. However, Clark (1960) has found that the replacement of a hydroxyl group with a methoxy group in the acid moiety of the molecule increases mutagenic activity, whilst the 1-2 double bond in the pyrrolizidine ring is also necessary for mutagenic activity. Clark (1959) has also found that the mutagenic activity is dependent on the whole molecule, since neither the acid nor basic moiety alone proved to be mutagenic. No data are present available on the occurrence of mosaics in *Drosophila* following treatment with pyrrolizidine alkaloids, but at least part of their action may involve both strands of DNA since it has been found that chromosome breaks are produced by the alkaloids.

To sum up, it is evident that a study of recovery from premutational damage will not only enable geneticists to determine the nature of this damage and the factors involved in its repair, but it will also enable a more effective comparison to be made of the steps involved in the production of radiation and chemically induced mutations. Also, investigations into the factors which influence recovery may be a step forward in the quest for directed mutation, whilst factors which modify mutagenic activity could have important practical implications in the treatment of cancer.

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