CHEMICAL MUTAGENESIS

By

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I. INTRODUCTION

Although geneticists had often speculated on the possibility of gene mutations arising as a result of reactions between the chromosomes and active chemicals, it was not until the discovery of the mutagenic activity of mustard gas in Drosophila by Auerbach & Robson in 1942 that intensive study of this field of work commenced. In the twenty years which have passed, a large amount of work has been done, initially on Drosophila and plants, subsequently on Neurospora, bacteria and viruses and more recently on mammalian cells. Much of the work on chemical mutagenesis has developed in association with attempts to use the so-called radiomimetic chemicals as effective inhibitors of cell division in malignant tissues. Equally important has been the stimulus provided by the somatic mutation hypothesis of the origin of cancer. Many chemicals have been tested successfully for mutagenic ability because they were already known to be either carcinostatic or carcinogenic in laboratory animals.

Originally the term radiomimetic was used in the sense that the chemical was able to simulate all the end effects of exposure to ionizing radiations. However, the term has more recently been applied to those compounds which satisfy the two criteria of ability to cause chromosome breakage and ability to bring about mutation, it being assumed that carcinostatic and carcinogenic ability are later manifestations of one or the other of these two primary causes. To designate a compound as radiomimetic in this sense in no way implies that its mode of action is essentially the same as that of radiation. Our experimental techniques are designed to detect mutations and chromosome breaks, but these are only the terminal stages of a series of events which may not have involved the same path under all circumstances. We do not know at what stage in the development of biological lesions the pathways from chemical mutagen and ionizing radiation meet. In this respect, it may be noted that some chemical mutagens show an oxygen effect, while others do not.

II. MUTAGENESIS AND CARCINOGENESIS

In so far as the relation between carcinogenesis and mutagenesis is concerned, it has long been realised that a purely chemical consideration of the aetiology of cancer is too narrow and restricted an approach. Although many carcinogens have been shown to be able to act under certain conditions as mutagens, in general there appears to be no quantitative parallelism between mutagenicity on the one hand and capacity to initiate, or inhibit, tumour growth on the other. There are several examples of chemicals which have mutagenic or chromosome breaking properties but which have not been found to be carcinogenic in laboratory animals. It may be that mutagenic properties are a necessary, but not a sufficient requirement for carcinogenic action. However, most hypotheses of chemical carcinogenesis fail to provide a satisfactory explanation for the fact that some very inert substances have been shown to be carcinogenic. The induction of tumours in rats by the subcutaneous implantation of plastic film can scarcely involve active participation of the implanted material in any chemical processes occurring in the tissues (Alexander & Horning, 1959).

Of the more recently discovered mutagens known to be carcinogenic, the pyrrolizidine alkaloids are of some interest. Esters of complex carboxylic acids with amino alcohols, these alkaloids were first detected in the plant Senecio, but have since been found in some of the Leguminosae and the Boraginaceae. They have been implicated as the toxic agents responsible for liver damage in grazing stock (Bull, Dick, Keast & Edgar, 1950) and have also been shown by Schoental & Magee (1957) to be specific liver carcinogens in the rat. Primary cancer of the liver in man is of rare occurrence in the peoples of Western Europe and North America, irrespective of whether they live in Europe, Asia or Africa. By contrast, it is remarkably common in the native populations of certain parts of Africa and South-East Asia (Berman, 1958). It has been suggested that the high frequency of liver cancer is related to the common use in those parts of the world of Senecio herbal remedies for a wide variety of ailments.

A number of the alkaloids have been tested for mutagenic properties in Drosophila and several have been found to be very active (Clark, 1958, 1960). Heliotrine is the alkaloid which has been most carefully studied so far. It is highly mutagenic when injected into adults or when included in the food. If present in the food at a concentration of about 0.001M, then a twenty-four feeding period causes as much genetic damage as exposure of the flies to more than 1,000 r. of X-irradiation. Prolonged feeding leads to sterility (Brink, 1963). A considerable proportion of recessive lethal mutations induced by heliotrine in Drosophila tend to be unstable and may revert back to wild-type. The alkaloid causes breakage in root-tip chromosomes (Anzani, 1961) and in marsupial chromosomes in vivo and in vitro (Clark, unpublished) but no information is available concerning its possible mutagenic activity in higher mammals.
III. CHROMOSOME BREAKAGE AND MUTATION

Because of the relative ease with which cytological observations can be made on cells of actively growing plant root-tips after they have been exposed to solutions of chemicals, much of the screening of chemical mutagens has been based on the ability to produce chromosome breaks. It is certainly true that most physical and chemical mutagenic treatments so far characterised are able to produce chromosome breaks, structural rearrangements and apparent point mutations. On the other hand, a number of compounds are known which are able to produce breaks but yet appear to be unable to bring about point mutations. In some instances, the apparent discrepancy may simply be due to specific sensitivity differences, for while breakage is often scored in plant material, mutations in the more restricted sense are usually scored in *Drosophila*, *Neurospora* or micro-organisms. Cortisone, penicillin and folic acid antagonists are able to cause chromosome breakage in the Allium test (Kihlman, 1960) but are not significantly mutagenic in *Drosophila*.

Many induced mutations are associated with structural rearrangements. They may involve position effects or gene mutations in the proximity of one of the breakage points. The proportion of mutations associated with complex structural changes such as inversions, large deletions and translocations varies according to the dose and nature of the mutagenic treatment. In general, fewer gross rearrangements are produced by chemical mutagens than by doses of X-rays giving comparable overall mutation rates. In some cases, it has been shown that the scarcity of interchromosomal changes is not due to a shortage of breaks. Dominant lethals, which have their basis in chromosome breakage, may actually be relatively more frequent after chemical treatment than after X-irradiation. That there is a qualitative difference in the nature of the breaks induced by chemicals and by radiation seems unlikely, for there is evidence to suggest that breaks induced by chemical treatment may participate with breaks induced by X-rays to give rise to structural changes. Auerbach and Silzynska (see Auerbach, 1960) have reached the conclusion that delayed opening of breaks is the main cause for the shortage of large rearrangements after chemical treatment. This would also explain the fact that mustard gas, in contrast to X-rays, produces translocations just as readily in late pre-meliotic cells as in post-melotic cells (Sonbati & Auerbach, 1960).

By the use of the ring chromosome technique, it can be shown in *Drosophila* that a substantial proportion of induced recessive lethal mutations are not associated with breakage events at all. For this reason, while it may be useful in practice to use chromosome breakage as a pointer to possible mutagenic activity, it should not be assumed that breakages and mutations are necessarily related.

IV. MUTAGEN SPECIFICITY

From the standpoint of achieving control over the mutation process, ionizing radiations have the great disadvantage that they are relatively non-specific. Apart from occasional non-random distributions of breaks and the failure of certain alleles to respond to the treatment at all, the usual genetic consequence of exposure to radiation is an overall, non-specific increase in mutation rate. Chemical mutagens, on the other hand, seem to offer the prospect of a greater specificity of action, perhaps even the induction of mutation at only one or two loci at a time.

Specificity of action of chemical mutagens appears in three ways. First of all, there may be a degree of specificity at the taxonomic level. A mutagen may be active in one species but not in another. Manganese chloride and nitrous acid are effective mutagens in micro-organisms but are inactive in *Drosophila*. Differences of this kind could have relatively trivial explanations in terms of accessibility, mode of administration, ease of passage across cell membranes or other phenomena. For example, the apparent difference could be the reflection of more subtle differences in the degree to which the mutagen is channelled into ineffective metabolic pathways, in the extent to which the biochemical lesions are subject to repair, or in the susceptibility of the genetic material itself.

A second kind of specificity is reflected by the different sensitivities toward the mutagen shown by various stages in germ cell differentiation. In the rat, triethylene melamine produces more dominant lethals in spermatids than in spermatogonial stages (Bateman, 1960). Mustard gas, on the other hand, produces its maximum effect on late spermatogonia. Differences of this kind may find their explanation along similar lines to those mentioned in connection with taxonomic specificity.

Of greater interest is the question whether, within any one particular cell stage, a mutagen is able to exert a preferential action on particular chromosomes or regions of chromosomes. In some cases, action of this kind could have a simple physical basis. Parts of chromosomes closest to the nuclear membrane during interkinesis might be exposed to higher concentrations of a chemical mutagen diffusing into from the cytoplasm. In plant material, there may be variability in the extent to which different chemicals cause breakage in heterochromatic regions, while in *Drosophila* sex-linked recessive lethal mutations sometimes tend to cluster in certain regions of the genetic map. Fahmy & Fahmy (1957, 1959) have analysed the genetic effects of a large number of carcinostatic and carcinogenic compounds synthesised at the Chester Beatty Research Institute and claim to have established significant differences in the qualitative effects of various mutagens. One of their criteria for specificity of action is the ratio of visible to lethal mutations produced in any given cell stage by the mutagen. Although they refer to the visible mutations as morphogenesis loci, this term does not appear to be very suitable. All genetic loci are morphogenetic. Hence the conclusion of the Fahmys that certain mutagens tend to induce mutations at morphogenesis loci cannot be sustained. However, it is possible that they have discovered that some mutagens tend to produce a high proportion of intragenic changes which may not be fully lethal and hence flies carrying such mutations would survive to be scored subsequently as
visible. Other mutagens, on the contrary, may produce a high proportion of deficiencies, which often have a recessive lethal effect. Differences in the extent to which repair is possible could give rise to change in the ratio of visible to lethal mutations observed in various cell stages.

The Fahmys have also claimed that some of their mutagens act on the gene loci which are stable towards X-irradiation. They report the discovery of more than 200 new loci on the X-chromosome of Drosophila melanogaster, new in the sense that spontaneous or radiation-induced mutations had not previously been recorded from those sites. The new loci are called beta loci and they are said to differ from the alpha loci by showing a high degree of radiation resistance as well as specificity of response towards particular chemical mutagens. Auerbach & Woolf (1960) have drawn attention to several unsatisfactory aspects of the work done by the Fahmys. Nevertheless, it should be noted that mutagen stability of the type attributed to the beta loci is well-known in bacteria, bacteriophage and Neurospora, and there is no obvious reason why the phenomenon should not be observed in higher organisms.

Some of the best examples of specificity of mutagenic response come from bacteria and viruses. The work of Freese and Benzer (see Freese, 1959) on the fine structure of the rII region in bacteriophage T4 is of particular interest. The rII region in the bacteriophage T4 consists of two cistrons designated A and B. Within each cistron there are many mutational sites, more than 250 in A but only about 48 in B. The following table, based on the data of Benzer and Freese, gives the percentage distribution of mutations induced at four of the sites in the B cistron is a series of experiments. Up to 85% of all the spontaneous mutations took place at site 117, yet none of the hydroxylamine induced mutations involve this site. Although the same number of tests was not carried out for each mutagen, nevertheless the data are sufficient to demonstrate a mutagenic specificity of a very fine order indeed, involving distinct mutational sites within a single cistron or physiological gene.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Site distribution of induced mutants</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spontaneous</td>
<td>360 N24 114 117</td>
</tr>
<tr>
<td>Spontaneous</td>
<td>% % % %</td>
</tr>
<tr>
<td>Nitrous acid</td>
<td>1 5 9 85</td>
</tr>
<tr>
<td>Ethyl methyl sulphonate</td>
<td>2 66 3 29</td>
</tr>
<tr>
<td>Hydroxyxylamine</td>
<td>10 45</td>
</tr>
<tr>
<td>5: bromouracil</td>
<td>25 75</td>
</tr>
<tr>
<td>5: bromodeoxyctydine</td>
<td>25 45</td>
</tr>
<tr>
<td>Ultra-violet light</td>
<td>4 55 7 34</td>
</tr>
</tbody>
</table>

Of some interest is the fact that certain chemicals may act as anti-mutagens, lowering either the so-called spontaneous rate or the rate induced by a chemical mutagen. Streptomycin is reported by Dubinin (1960) to lower the spontaneous mutation rate in Drosophila melanogaster. Guanosine lowers the spontaneous mutation rate in Escherichia coli and also reduces the mutagenic activity of caffeine and theophylline.

Reference has already been made to the different responses which various cell stages may give to a particular mutagenic treatment. Effects of this kind are well-known and in Drosophila are analysed by the brood fractionation method. Treated males are mated to successive groups of fresh virgin females at regular time intervals. The progeny in each successive brood represent male germ cells which were at progressively younger stages at the time the mutagenic treatment was applied. Identification of particular stages such as spermatids, spermatocytes and spermatagonia can be obtained, but only by controlling the experimental conditions very carefully so as to achieve an even rate of sperm maturation and utilisation. Transition from pre-meiotic to post-meiotic stages may be identified by scoring the progeny for induced crossing-over or for induced non-disjunction of the X and Y chromosomes, while clusters of identical crossovers or of mutants at similar loci serve to identify other stages. Cramer & Schmes (1962) have used tritiated thymidine for the radio-autographic estimation of the rate of sperm maturation in Drosophila.

The brood pattern of sensitivity to a particular chemical mutagen may vary according to the species. Thus, triethylene melamine has its maximum effect on late spermatogonial cells in Drosophila but on post-meiotic stages, probably spermatids, in the mouse (Reddi & Auerbach 1961, Bate­man 1960). The sensitivity pattern may also depend on the mode of access to the mutagen. Formaldehyde present in the food of larval Drosophila exerts its effects mainly on pre-meiotic stages, whereas when injected into adult males its effect is mainly on mature spermatozoa. When fed to adult males, it is only weakly mutagenic. Alderson (1961) has found that the mutagenic action of formaldehyde in the food of the larval stages is dependent on the presence of adenylue acid. The latter alone is not mutagenic. It seems probable that in the case of formaldehyde mutagenesis, there is a real difference between the pathways of mutagenic action in larva and adult. One of these pathways probably involves interference with the incorporation of adenine into the DNA at the time of chromosomes replication.

V. THE MUTATION PROCESS

A correlation between spontaneous mutation and active cell growth has often led to the assumption that cell division is required for mutation. Strauss (1960) goes so far as to say that there can be no mutation without DNA replication. While there is certainly good evidence that some mutations result from errors in gene replication, it is unlikely that this is the only way in which mutation can occur. Some years ago, Novick & Szilard (1951) found that the spontaneous mutation rate to T5 phage resistance in E. coli growing in a chemostat was independent of growth rate. It was, however, time dependent. This would suggest that mutation involved some change in an existing gene molecule, rather than an error during replication. However, the interpretation of data from the accumulation of bacterial mutants in a chemostat may be difficult.
Kubitschek (1969) found that if glucose is used to limit growth, the accumulation of caffeine-induced mutants is proportional to the growth rate, thus supporting the error hypothesis. In a later paper (Kubitschek & Bendigkeit, 1961), he finds that if tryptophane is used to limit growth, then both spontaneous and induced mutation rates are found to be independent of growth rate. Since the kinetics of expression of mutation seem to be dependent on the nutrient used to limit growth rate, rather than on the nature of mutagenic treatment, it seems unlikely that in this case the process of mutation can be regarded as an error in template replication. Kubitschek emphasizes the distinction to be made between mutation, the induction of which he suggests is independent of the growth rate of the culture, and the physiological expression of the mutation, which may be affected by the nature of the growth-limiting factor employed.

The work of Altenburg & Browning (1961) and of Muller, Carlson & Schalet (1961) argues strongly in favour of a chemical change in an already existing gene. The work is based on a study of the proportion of mutations in Drosophila found to be fractional, i.e., involving only part of the body of the mutant individual. Multiple markers were used which enable the mosaic or fractional individuals to be scored reliably. According to the Watson-Crick model of DNA, a mutagen which causes an error in gene replication in a diploid embryo should produce a mosaic individual.

More than 50% of spontaneous and chemically induced mutants were found to be fractional, compared with only about 7% of X-ray induced mutants. Most of the spontaneous fractional mutations occur in the spermatid stage, i.e., in post-meiotic chromosomes. If they are delayed effects leading to errors of replication of DNA at the first division of the fertilized egg, one would expect them to be quarter fractional. In fact, they are mostly one-half effects, so that they can have arisen only as a permanent change in one of the strands of an already existing double strand DNA molecule of the post-meiotic chromosome.

How the spontaneous and chemically induced fractional mutants are produced is at the moment only a matter for speculation. It seems unlikely that a chemical mutagen would bring about a direct chemical change in a base, the latter remaining attached all the while to the DNA strand. If, on the other hand, the chemical reaction involves removal of the base, it would tend to be restituted by the complementary base in the other strand, thus giving no genetic change, unless double breakage had caused rotational substitution. But in that case, the mutation would no longer be fractional. Substitution of base analogues might produce mosaic fractional mutants, but then it would be necessary to assume that the chemical mutagen acts by bringing about a change in the pool of purine or pyrimidine bases, an altered analogue subsequently being incorporated during replication. In any event, this explanation would not apply to the half-fractional mutations induced in post-meiotic chromosomes in Drosophila germ cells.

There is good evidence from bacterial and phage genetics that base analogues may induce mutations and, in some cases, incorporation of the analogue into the DNA has been demonstrated (Rudner, 1961). It is generally assumed that the mutagenic action is directly related to the incorporation of the base analogue. On the other hand, it is also known that in the T phages, for example thymine can be replaced almost completely by bromouracil without preventing the production of viable phage by lethal mutation. Transforming principles may still retain their activity after being heavily substituted with bromouracil. It is difficult to avoid the conclusion that the incorporation of a base analogue is not the only factor involved in the mutagenic activity shown by such compounds.

The mode of action of a chemical mutagen in higher organisms becomes even more difficult to visualise in the light of recent cytological evidence in favour of a multi-stranded structure of the chromosome. It is customary to regard the Watson-Crick model of DNA as being also a reasonable model of the structure of the chromosome as a whole, notwithstanding the obvious fact that these are two very different structures of quite different orders of magnitude. Since there are good grounds for assuming that in some cases a single chromatid may contain at least sixty-four strands of DNA double helices (Steffenson, 1961), it is not easy to see how chemical mutagens produce any genetic effects at all.

VI. CONCLUSION

In conclusion, what generalisations can be made about chemical mutagens on the basis of the present state of knowledge? Clearly, they may be as potent as ionizing radiations in their effectiveness in bringing about genetic change. Sensitivity differences are striking. There are clear indications of specificity of action, so that an altered spectrum of genetic effects may be obtained by the use of a variety of mutagens. Many of the induced mutations are fractional and can give rise to delayed manifestation or the production of mosaic individuals.

On the other hand, no generalisations can be made about the mode of action of the mutagenic and chromosome breaking chemicals. Dozens of different substances have been screened and found to be active. A list of some of them would include alkylating agents, nitrogen mustards, formaldehyde, phenol, glycol, ascorbic acid, acridines, basic dyes, certain sugars, quinolines, menthol, insecticides, urethane, barbiturates, organic arsenicals, peroxides, cyanides, the salts of heavy metals, chelating agents, sulphides, urea, alkaloids, various natural oils such as lavender and eucalyptus, penicillin, salicylates and caffeine. Where is the common factor? Most cells will show some kind of cytological or genetic response to X-irradiation and it would be reasonable to assume that the biochemical events following the absorption of radiant energy are in most cases essentially the same. No such simplifying assumption can be made for the chemical mutagens.

One final point to be mentioned concerns the possibility that some of the chemicals in common use in food processing or therapeutics may have mutagenic properties in man. Much time is devoted to the discussion of the genetic significance.
of relatively small increases in the levels of background radiation to which human populations are exposed, yet little interest is shown in the possibility that genetic damage may result from mass exposure to chemical mutagens. Caffeine is a powerful mutagen in bacteria and fungi. It is a weak mutagen in Drosophila. The only published experiment describing an attempt to assess its mutagenicity in the mouse gave a negative result. What conclusions can be drawn as to the possible genetic consequences of the daily cups of tea and coffee? It is to be hoped that the current interest in the ability of drugs, such as thalidomide, to produce developmental malformations or phenocopies may ultimately stimulate interest in the desirability of adequate genetic screening of new therapeutic agents before they are released for general use.

VII. REFERENCES

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