MUTAGENS

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INTRODUCTION

The subject of this review, the induction of mutations by chemicals, was greatly stimulated and helped by wartime research. In this it resembles radiobiology, the investigation of antibiotics and other fields of scientific enquiry. It is a field in which much is uncertain and much remains to be investigated. From the point of view of clinical medicine mutagens are of interest, first because some of them have found application in the palliative treatment of leucaemias and allied diseases and secondly, because they should be used with great care, as they may be carcinogenic.

A mutation is a discontinuous change in quality of offspring which can be inherited. Authors' statements that mutations have been induced have been accepted in this review except in those cases where only chromosome breaks have been induced and described as mutations. In some cases, particularly in bacteria, the changes may be induced adaptations. All the changes probably have a chemical basis in that a gene governing the production of an enzyme or enzymes is destroyed or modified. The induction of a mutation in most cases consists of specific and limited destruction or change of a part of the system which is treated; in this mutagenesis resembles vesication.

No attempt is made to give a complete list of substances which have been found to be mutagenic; a list of substances tested in *Drosophila* is given by Herskowitz (78). The techniques used have been discussed by Auerbach (5).

MUTAGENIC ACTION OF VESICANTS

Mutations were first induced experimentally by exposing *Drosophila* to X-rays by Muller (105) who developed ingenious techniques for detecting induced muta-

tions. Later, mutations were induced with ultra-violet light (100), but Muller (106) reviewing the field in 1941, was able to say "to date, radiation has stood as the only external agent, by the artificial application of which, mutations can be produced in abundance and with predictable frequency". But in the same year, Auerbach and Robson found that mustard gas ($\beta\beta'$-dichlorodiethylsulphide) is comparable with X-rays in its capacity to produce mutations and chromosome rearrangements. The publication of these findings was delayed until the war was over (9, 10) but the findings have been frequently confirmed (see 5). There had, however, been earlier descriptions of chemical mutagenesis. For example, Lobashov (98) had observed an increase in mutation rate in *Drosophila* on treatment with ammonia and Auerbach and Robson (10) also found ammonia to have slight mutagenic activity. This and other early work on chemical mutagenesis can be appraised better since the discovery of really potent mutagenic agents.

The idea of testing vesicants as mutagens was conceived by Dr. J. M. Robson, who was struck by the similarity of mustard gas burns and X-ray burns, and by the fact that mustard gas and X-rays both inhibited mitosis in hormonally stimulated vaginal tissue. Other vesicants related to mustard gas which were also found to be potent mutagens included the nitrogen mustards, methyl *bis*(β-chloroethyl)amine (HN2), *tris*(β-chloroethyl)amine (HN3), *bis*(β'-chloroethyl-β-mercaptoethyl) ether, ($(ClCH_2CH_2SCH_2CH_2)_2O$) and the weak natural vesicant, mustard oil or allyl*iso*thiocyanate. Although most of the vesicants were found to be mutagenic, the very potent vesicant lewisite (β-chlorovinyldichlorarsine, $ClCH:CHAsCl_2$) did not induce mutations. In the reviewer's opinion the lack of mutagenic activity of lewisite might be due to its extremely high chemical reactivity, particularly with water, so that few lewisite molecules could penetrate to the cell nuclei without hydrolysis into the toxic lewisite oxide. For this reason it would be of interest to examine the mutagenic action of lewisite on dry spores of a suitable organism. The differences between the effects of vesicants and radiation are not negligible (7).

Many substances have given less consistent results than the vesicants. For example, Hadorn and Niggli (73) induced mutations by immersing dissected gonads of *Drosophila* in solutions of 0.01% phenol before transplanting the treated gonads into fresh larvae. The offspring derived from the phenol-treated gonads had increased incidence of visible, semi-lethal and lethal mutations but treatment with colchicine, a known mitotic inhibitor, had no effect on the incidence of mutants. In later experiments with the same technique and by exposure to aerosols (Hadorn, Rosin and Bertani, 74) no mutations were induced by phenol, quinone, picric acid or cresol. At the same time, no induced mutations were observed when larvae were fed on a diet containing phenol, or were injected with phenol.

In this case workers were not always able to repeat the experiments in which mutations were induced with phenol. Another example of the difficulty and uncertainty of work in this field is shown in experiments with carcinogenic aromatic hydrocarbons, and aromatic amines. Thus Demerec (48) showed that the incidence of sex-linked lethals in *Drosophila* males was increased by treatment

with aerosols of the carcinogenic compounds 1:2:5:6-dibenzanthracene, 20-methylcholanthrene, 3:4-benzpyrene and *p*-hydroxyazobenzene but not by treatment with the non-carcinogenic substances anthracene, phenanthrene, pyrene, azoxybenzene or diethylaminoazobenzene. Increased mutation rate was also obtained with two substances which are doubtful carcinogens—aminoazobenzene and sodium desoxycholate (cf. 14). Later, however, these observations on mutagenesis by aromatic carcinogens could not be repeated in the same laboratory (51), or in other laboratories (cf. 34).

A possible explanation of these anomalies may lie in the fact that the substances investigated must be metabolized in the organisms to produce the proximate mutagen. The essential specific metabolic process might be dependent on contaminating symbiotic organisms, present at the time when positive results were obtained but absent when no increased mutation rate was observed. This explanation is less plausible in the case of the mutations induced by immersing gonads in phenol solution than in the experiments in which insects were exposed to aerosols of carcinogenic compounds.

RESEMBLANCE BETWEEN MUTATIONS AND THE EFFECTS OF IRRADIATION

The discovery of powerful, effective mutagens was followed by the investigation of hundreds of substances for mutagenic action and of the effect of mutagens on other organisms; but perhaps the most interesting development has been the study of the correlation between power to induce mutations and other biological and chemical changes. One of these effects which has generally been found to be closely associated with mutagenesis is the production of chromosome breaks. The production of such effects by X-rays had been described (104) before Muller observed the mutagenic action of X-rays. The corresponding effect of mustard gas on chromosomes of *Tradescantia* was described first by Koller, Ansari and Robson (92) and later in more detail (44). The production of chromosome breaks or other damage is a visible change which is perhaps analogous to the physiological change manifested as a mutation. The similarity of the effects of ionising radiations and of certain chemicals has led to the use of the term 'radiomimetic' to be applied to such effects and to substances which produce them. Examples of such actions which have already been mentioned are vesication, induction of mutation and causation of chromosome breaks.

The application of the term 'radiomimetic' is not meant to imply that the mechanism of action of radiations and of chemical agents are the same. Although radiations probably act by liberation of free hydroxyl or other free radicals in the system which then produce chemical changes, it is unlikely that most of the radiomimetic drugs operate through such a mechanism. The vesicant effect of radiations and vesicants is also unlikely to be due to action on chromosomes. The term 'radiomimetic' is therefore used in a very wide sense (some workers might consider the term to be used loosely) and is analogous to such terms as 'narcotic' or 'cathartic.'

Effects which can be evoked by radiations and by some chemical agents are listed in Table I (from 26). The only known chemical substances which produce

TABLE I

Both ionizing radiations and radiomimetic drugs

1. Inhibit growth of tumours or of the whole body.
2. Induce cancer at site of action.
3. Produce chromosome damage.
4. Produce mutations.
5. Cause delayed death with similar post mortem changes.
6. Cause erythema and inflammation.
7. Destroy viruses.
8. Depolymerize nucleic acid *in vitro* and also *in vivo*.
9. Destroy white cells of the blood, causing leucocytopenia.
10. Reduce blood clotting ability, probably owing to reduction of the number of circulating thrombocytes.
11. Cause local greying of hair.
12. Inhibit the development of immunity which involves the production of antibodies when an antigen is administered.
13. Destroy complement (a normal blood constituent necessary for lysis of foreign red blood cells).
14. Inhibit sulphydryl enzymes, such as triosephosphate dehydrogenase.
15. Produce blisters on skin.
16. Cause haemoconcentration owing to withdrawal of water from blood.
17. Cause nausea and vomiting ("radiation sickness") and lesions in the intestinal mucosa.
18. Cause delayed hyperglycaemia.
19. Produce foetal abnormalities in pregnant animals.
20. Cause a negative nitrogen balance, owing either to increased breakdown of protein or to decrease in protein synthesis.

all these effects are some of the vesicants—the aliphatic nitrogen and sulphur mustards. The absence of mutagenic action of the powerful vesicant lewisite has been mentioned, and many of the known chemical mutagens are not vesicant. These relationships and differences are, however, of great interest and in some cases of clinical importance. For example, of all the substances of this type which have been investigated from the point of view of inhibition of tumour growth (cf. 70) only a few have found clinical application. An aromatic nitrogen mustard, *p-bis*(chloroethyl)amino phenylbutyric acid, may be of value for patients suffering from lymphatic leucaemia (Galton, Personal Communication). The nitrogen mustards HN2 (64), HN3 (144) and β *bis*(chloroethyl)aminonaphthalene (60), as well as triethylene melamine (114), are used in the treatment of Hodgkin's disease. Myleran or 1:4-dimethanesulphonyloxybutane, an alkylating agent of a different type, has found application in the treatment of myeloid leucaemia (61).

MUTAGENIC ACTIVITY OF CYTOTOXIC ALKYLATING AGENTS

In the study of the biological effects of compounds related to the nitrogen mustards many investigators found that substances with two or more active centres (*i.e.*, chloroethyl groups) were more effective than related compounds with only one such group. Because of this Goldacre, Loveless and Ross (63) suggested that these substances owed their power to induce mutations and chromosome damage to their ability to cross link the chains of some nuclear constituent.

Following this suggestion known cross-linking agents such as butadiene diepoxide (21) and triethylene melamine (20) were tested and found to be mutagenic. Although such compounds are generally more active than the corresponding monofunctional compounds, increased mutation rates have been observed with the monofunctional alkylating agents including ethylene oxide (20, 119), ethyleneimine (20), β-chloroethyl-ethylsulphide (8), monofunctional chloroethylamines (94), acylethyleneimines (76), diazomethane (86, 118) and dimethyl sulphate (117).

The nitrogen mustards and the compounds derived from them such as the aromatic chloroethylamines (19), the diepoxides (77), the polyethyleneimines (76) and the dimethanesulphonoxyalkanes (72) are all substances which can alkylate amino groups or esterify anions or acid radicals. The cytotoxic alkylating agents are able to alkylate the carboxyl groups of proteins or the phosphoric acid residues of nucleic acids. In many cases the biological activity has been shown to be paralleled by the chemical reactivity as measured by the ability to react with acid groups (cf. 124). This has been shown most clearly with the derivatives of phenyl *bis* (chloroethyl)amines (124) and the dimethanesulphonoxyalkanes (72).

THE RELATIONSHIP BETWEEN MUTAGENS AND CARCINOGENS

Many of the mutagenic cytotoxic alkylating agents have been shown to be carcinogenic. Tumours have been induced in animals with nitrogen mustards by Boyland and Horning (29), Heston (79) and Griffin (66); with mustard gas by Haddow (personal communication) and Heston (80); with aromatic nitrogen mustards, particularly 2(*bis*-chloroethyl)naphthylamine (69); with diepoxides (77), polyethyleneimines (76), and the monofunctional acyl ethyleneimines (76). Thus compounds of each of the known types of cytotoxic alkylating agents which produce mutations have been shown to induce cancer in animals. The carcinogenic action of nitrogen mustard is reduced by irradiation, which is itself carcinogenic (81). There is however no quantitative parallelism between mutagenic power, induction of chromosome damage, inhibition of tumour growth or carcinogenic activity. This is not surprising as, in the reviewer's opinion, it is generally impossible to express carcinogenic activity in quantitative terms and the carcinogenic activity can only be shown in vertebrates while mutagenic power is usually measured in insects, moulds, bacteria or viruses. In general mutagens of the type of the cytotoxic alkylating agents are carcinogenic.

There are a few other types of compounds which have been found effective as mutagens and carcinogens, including urethane and propyl- and isopropylcarbamates (discussed later). Styryl 430 (2(*p*-aminostyryl) 6-*p*-acetylaminobenzoylaminoquinolinemethoacetate) has been shown to be carcinogenic by Browning (32) and mutagenic for *E. coli* (96) but not mutagenic in *Drosophila* (Fritz-Niggli, Personal Communication). Desoxycholic acid is possibly carcinogenic (14) and is mutagenic for *E. coli* (145).

There are however some effective mutagens which have not been proven to be carcinogenic. Among these are the aldehydes. Formaldehyde is mutagenic for

Drosophila when given in food (6, 87, 116) but it has not been effectively tested for carcinogenic action. Acrolein, an active mutagen for *Drosophila* (120), was found to be very toxic for mice and the maximum tolerated dose (0.2 mg. per mouse weekly) did not induce sarcomata (131). In many cases information is lacking; for example phenol which has sometimes been found mutagenic and manganous chloride which induces mutations in *E. coli* (50) do not seem to have been tested for carcinogenic activity.

There are many known aromatic carcinogenic compounds including the polycyclic hydrocarbons and heterocyclic compounds such as the benzacridines, benzcarbazoles and benzothiophenes. All the compounds of this type are only very slightly soluble in water so that their application to the relatively simple systems generally used to test for mutagenic power presents difficulties. The induction of tumours is also a slow process; the most rapidly acting carcinogen, 9:10-dimethyl-1:2-benzanthracene, rarely produces tumours in three months. (The actively mutagenic vesicants usually take twelve months to induce cancer.)

Because of the possibility that cancer arises as a somatic mutation Auerbach (4) tested 1:2:5:6-dibenzanthracene on *Drosophila* (before the clear cut results with vesicants had been obtained) and observed no increase in mutation rate. Demerec and Hollaender (cf. 47) tried to induce mutations by raising flies for twelve generations on food containing 1:2:5:6-dibenzanthracene with and without exposure to ultraviolet light; these experiments were inconclusive. Demerec (48) then used the technique of exposure of flies to aerosols, which Auerbach and Robson (9, 10) had found so effective with vesicants. In the first experiments (47) the incidence of sex-linked lethals was 0.98% in the offspring of flies treated with 1:2:5:6-dibenzanthracene in sesame oil compared with 0.28% in the controls treated with sesame oil. Later (48) mutations were obtained with other hydrocarbons and aromatic amines but later still (51) these results could not be repeated (see p. 346).

Another group of carcinogenic compounds consists of the aromatic amines as exemplified by the aminoazobenzenes, aminostilbenes (68), aminobiphenyls, β-naththylamine and acetylaminofluorene (17). Some of these were found to be active mutagens by Demerec (48) at one time, but not later (51). These aromatic amines are only very slightly soluble in water, react very slowly and produce biological effects very slowly. The aromatic carcinogens show considerable specificity for animal species and sites, and their actions are dependent on dietary and hormonal influences. Thus, β-naphthylamine induces cancer of the bladder in man with a mean induction time of 17 years (127), and dogs (85) with a mean induction time of several years but does not induce cancer in mice, rats or rabbits. Acetylaminofluorene produces cancer of the liver when fed to normal rats (17) but does not do so in thyroidectomised rats (18) or in rats treated with antithyroid drugs such as thiouracil (38). It produces liver cancer more rapidly if combined with oestrogens (38). It induces cancer of the bladder when injected into mice (3) or fed to rats in a diet containing hydrolysed casein and *dl*-tryptophane but not when fed to rats on a normal diet (56).

During the last few years, work on the mechanism of the carcinogenic action

of aromatic amines (24, 141) has indicated that these amines may not be active as such, but only after conversion, by metabolic oxidation, to *ortho*-aminophenols. The first clear example of this was with β-naphthylamine, one metabolite of which, 2-aminonaphthol-1, was shown to be probably carcinogenic by Hueper (84) and more recently by Bonser *et al.* (24) under conditions in which β-naphthylamine was inactive. The latter workers have also shown that the isomeric 1-aminonaphthol-2 is also carcinogenic. This hypothesis of the mechanism of action of carcinogenic aromatic amines is still under investigation and it is not known with certainty if it applies to all such compounds. It would be of great interest and value to know if such *ortho*-aminophenols derived from carcinogenic compounds are mutagenic. There are differences in the ability of different mammals to convert aromatic amines to *ortho*-aminophenols; dogs which develop cancer of the bladder on treatment with β-naphthylamine convert this substance into 2-aminonaphthol-1 while rabbits convert it mainly into 6-aminonaphthol-2. The activity of other carcinogens is dependent on the species of animals and on their hormonal and dietary condition and the carcinogenic action is always very slow. If such differences in carcinogenesis exist with different vertebrate animals then it is not surprising that these substances do not produce genetic changes in insects. Although many carcinogens have organ specificity (*e.g.*, the azo dyes induce cancer of the liver) only the oestrogens and zinc salts show any specific carcinogenic action on the testis or ovary, and these carcinogens do not appear to have been tested for mutagenic activity. If a substance is to produce genetic mutations it must act on the gonads.

Some tests on the mutagenic activity of carcinogenic hydrocarbons in mice have given positive results although the statistical evaluation of mutation rates in mice is extremely difficult. Strong (133, 134) injected many generations of specially bred hybrid mice with methylcholanthrene and observed many variants within the line and in crosses with other inbred mice. The abnormalities, which were proved to be mutations by breeding tests, included many types of colour change such as white ears, patterned mice, pink, albino and black coats. In the untreated mice of the colony only 8 mutations have been observed among 210,000 mice giving an estimated mutation rate of 1 in 26,250 as compared with a rate of 1 in 557 among the treated mice. The treatment with methylcholanthrene induced an apparent "unstable genetic state" and variants such as increased body weight, increased number of the litter and *situs inversus* which were not proved to be mutations appeared. These mutation rates are comparable with the rates obtained by Russell (125) on the induction of mutations at 7 specific loci in mice by X-rays; 48,007 mice irradiated with 600 r. gave 54 mutants (1 in 890) while 2 mutants were found in 37,808 control mice.

Carr (39) working with fewer animals than Strong produced mutations by injecting 83 mice with 1:2:5:6-dibenzanthracene over four continuous generations. Seven variants were obtained among the offspring of these mice of which four were proved to be recessive gene mutations. In this experiment the treatment with the carcinogenic hydrocarbon must have raised the mutation rate but it is impossible to assay the effect quantitatively.

EFFECTS OF MUTAGENS ON CHROMOSOMES

Other studies on the effects of aromatic carcinogenic compounds on mammals which are of interest have been made by Koller (91) who examined the effects of radiation (52), nitrogen mustard (28), urethane (30), carcinogenic aminostilbenes (91) and carcinogenic hydrocarbons (91a) on the chromosomes of the Walker carcinoma 256 growing in rats. All these agents cause similar (but not identical) types of injury on the chromosomes of the carcinoma. Irradiation with 200 r. or dosing with 1 mg. per kg. body weight of nitrogen mustard (HN2) produce about the same amount of damage, but whereas the maximum effect following irradiation is seen after 12 hours, the proportion of cells injured by nitrogen mustard increases until 144 hours after injection (93). Cells at the end of the resting stage seem to be most sensitive to X-rays and least sensitive to nitrogen mustard. In addition to the difference in the time at which X-rays and nitrogen mustards produce their effects on chromosomes they appear to act on different regions of chromosomes (59, 121). Nitrogen mustards, diepoxides and tertiary butylhydroperoxide acting on *Vicia* or *Oenothera* (112, 121) produce relatively more breaks in the short chromosomes, and more breaks are produced in the heterochromatic regions of the chromosomes than is the case with X-rays. Urethane and X-rays both produce chromosome breaks which are almost randomly distributed along the chromosomes. This difference may be due to difference in accessibility of sites; the radiation will produce radicals throughout the cell but chemicals will be limited by diffusion (123).

When injected subcutaneously into tumour-bearing rats, the carcinogenic hydrocarbons 1:2:5:6-dibenzanthracene and 3:4-benzpyrene and the carcinogenic 4-dimethylaminostilbene derivatives (91) all produce effects on the chromosomes of the Walker carcinoma similar to those produced by X-rays (52). The effects are produced more slowly than is the case with nitrogen mustard. The carcinogenic 2′-methyl-4-dimethylaminostilbene appeared to be much more active in producing chromosome damage than the isomeric non-carcinogenic 4′-methyl-4-dimethylaminostilbene. The fact that the carcinogenic hydrocarbons and aminostilbenes produce chromosome breaks in rat tissues indicates that these carcinogens are probably mutagenic in the rat—an animal in which these compounds induce cancer.

In the reviewer's opinion the correlation between carcinogenic and mutagenic action is reasonably close, considering that the two effects are usually tested on different species or quite widely differing types of organism and that the assays of both mutagenic and carcinogenic activity are difficult. There are, however, opponents of this view and Burdette (33, 35) has drawn attention to discrepancies. The observation that 1:2:5:6-dibenzanthracene produces "tumours" in *Drosophila* but no mutations (34) would have more significance if the tumours in *Drosophila* were true cancers. On the whole the evidence is not in disagreement with the hypothesis that cancer is a somatic mutation which can be brought about by such agents as can induce genetic mutations.

PEROXIDES

Since 1939 great progress has been made in research into the mechanism of action of radiations. Like work on other mutagens, this has been, to some extent, a by-product of wartime and post-war research connected with atomic energy. One line of radiobiological research has been the study of the genetical and cytological changes which have been mentioned. Another approach has been the study of chemical changes caused by radiation which are greater in the presence of oxygen and reduced in the absence of oxygen or the presence of reducing agents. Many of the biological effects of ionising radiations are similarly dependent on the oxygen content of the organisms. Thus the LD 50 for irradiation for rats exposed to X-rays is doubled if they are exposed in a mixture containing 5 % oxygen instead of normal air (54). The effect of X-rays in producing chromosome breaks in *Vicia faba* is about three times as great in oxygen as it is in nitrogen (136).

The effect of oxygen in increasing radiosensitivity might be due to a change in the chromosomes or to the oxygen increasing the formation of the injurious product; there are many experimental findings in agreement with the second possibility (cf. 65). When water is exposed to α-radiation hydrogen peroxide is formed whether oxygen is present or not but with X-rays detectable hydrogen peroxide is formed only when dissolved oxygen is present (23). Further study of the effects of radiations has shown clearly that X-rays, α-rays or ultraviolet light decompose water to give free H and OH radicals (143). These radicals can then react with each other to give hydrogen and hydrogen peroxide or water, or with other substances present such as benzene which is oxidised to phenol (130), vinyl or acrylate derivatives which are polymerised (43), or nucleic acid which is depolymerised (46, 126, 135). Thus although ionizing radiations are physical mutagens they probably produce their biological effects by forming free hydroxyl and hydrogen radicals and possibly hydrogen peroxide within cells.

If *Staphylococcus aureus* is grown in nutrient broth which has previously been irradiated with ultraviolet light the rate of development of mutant forms, unable to ferment mannitol, resistant to penicillin or resistant to streptomycin, is increased (132). Later it was shown that irradiated culture medium could induce mutations in *Neurospora crassa*, the nature of which could be proved by breeding experiments (140). The mutagenic action of the irradiated medium was increased by cyanide, and mutations were induced in *Neurospora* with hydrogen peroxide and potassium cyanide; cyanide was added in these experiments as an inhibitor of the catalase present in the organisms. These results are in agreement with the hypothesis that some of the effects of irradiation are due to the production of hydrogen peroxide (or other peroxide) which then acts as a mutagen.

Later work showed that the mutagenic action of irradiated media could not be entirely due to hydrogen peroxide; the effect might be due, however, to some organic peroxide. With this possibility in view, Dickey, Cleland and Lotz (53) compared the mutagenic action of some peroxides on *Neurospora*. Hydrogen peroxide alone was not very active but mixtures of hydrogen peroxide and for-

maldehyde or acetone were much more effective. Under these conditions tertiary butylhydroperoxide, hydroxymethyltertiary butylhydroperoxide and the peroxide derived from di-isopropyl ether were all very effective mutagens while phenol formaldehyde, potassium permanganate and tertiary butyl alcohol tested under the same conditions were all inactive.

Chromosome breaks have been produced in dry *Tradescantia* pollen grains by exposure to an atmosphere of 65% oxygen for one hour (42). Such treatment caused the same degree of damage as exposure to 350 r. under normal conditions. Thus mutation is a possible effect of oxygen poisoning. The active peroxides may function by liberation of free hydroxyl radicals or by direct oxidation of some cell constituent.

ALDEHYDES

Formaldehyde was first investigated by Rapoport (116) who obtained 5.9% of sex-linked lethals in *Drosophila*, the larvae of which had been fed on a diet containing formaldehyde. Other workers obtained 5.7% (87) and 5.0% (6) but the exposure of adult flies to the vapour of formaldehyde did not induce mutations. The effect of the formaldehyde is therefore due to the aldehyde changing some constituent of the food, or to some product of formaldehyde formed in the diet. If the aldehyde were oxidised to the corresponding peroxide, dihydroxymethyl peroxide, the effect would be analogous to that found with other organic peroxides (53). Rapoport (118) however considers that the formaldehyde reacts with an essential amino group of genes and this appears to be the most probable mode of action. In further investigations he found acetaldehyde was also mutagenic but higher saturated aldehydes were inactive (120). On the other hand unsaturated aldehydes such as acrolein, citronellal and propiolaldehyde were much more active than the saturated aldehydes. Some of these aldehydes are of special interest as they may occur in nature and so act as endogenous mutagens; citronellal is present in the eucalyptus and lemon, formaldehyde is possibly an intermediate in transmethylation reactions and acrolein occurs in tobacco smoke. Formaldehyde and citronellal do not appear to have been tested adequately for carcinogenic action.

URETHANE

Before mutations had been artificially induced, Hawkins and Murphy (75) noticed that urethane (ethyl carbamate) had a leucopenic action in rabbits. In 1943 Nettleship and Henshaw (107) discovered that urethane induced cancer of the lung in mice and Oehlkers (111) found that it caused chromosome breaks in *Oenothera*. Haddow and Sexton (71) observed inhibition of growth and histological changes in tumours of rats treated with urethane and later the drug was introduced into medicine for the treatment of chronic myeloid leucaemia (113). Urethane induces mutations in *Drosophila* (138, 139) and in bacteria (49, 96), but not in *Neurospora* (86).

Of the alkyl carbamates ethyl carbamate is the most active in inducing cancer in mice (95) or mutations in bacteria (49, 96). Isopropyl carbamate has some

action but methyl carbamate and *iso* amyl carbamates are inactive either as mutagens or carcinogens. The effects are not produced by other narcotics such as barbiturates (95) and lung tumours are induced in mice with doses which are less than the dose necessary for anaesthesia. Urethane in relatively high concentration inhibits the growth of *E. coli* and this inhibition is neutralised by 2:6-diaminopurine and 2:6-diaminopurine riboside (128, 129) which are purine antagonists and inhibitors of tumour growth (57). Diaminopurine, however, does not neutralise the growth-inhibiting or chromosome-breaking effects of urethane on the Walker carcinoma in rats (30). Urethane produces many different effects on respiratory enzyme systems but McKinney (101) found that ethyl carbamate was much more effective than other simple alkyl carbamates as an inhibitor of transmethylation reactions such as the synthesis of creatine. The synthesis of thymine, an essential constituent of desoxyribonucleic acid, might involve a similar transmethylation process. For this reason the effects of thymine and urethane on the chromosomes of the Walker rat carcinoma were studied (30). Administration of thymine reduced the chromosome-damaging action of urethane and actively increased the rate of recovery from the effects of urethane.

PURINES

The mutagenic action of caffeine and theophylline has been shown with the ascomycete *Ophiustoma* (89) and *E. coli* (49). Mutations in *E. coli* are also produced with paraxanthine, theobromine, 8-azaguanine tetramethyluric acid and adenine (109). Kihlman (89) studied the effect of twenty four purine derivatives on the chromosomes of *Allium* and *Pisum* tissues. The most active compounds were 8-methylthiocaffeine, 8-chlorocaffeine, 8-ethylthiocaffeine, 8-methylaminocaffeine, 8-methoxycaffeine, 8-allyloxycaffeine, 8-ethoxycaffeine, tetramethyluric acid and caffeine. These compounds are all active solubilizing substances as indicated by their ability to dissolve polycyclic hydrocarbons (142) or the dye congo rubin, and the ability of the different purines to break chromosomes was in general paralleled by their solubilizing power. These purines may actually dissolve the material of the chromosomes and thus cause breakage and deficiency. If this were the mode of action in production of mutations it is difficult to see why the effect of caffeine should be neutralised by small amounts of guanosine (110). The mutagenic purines may be producing their effects by acting as antimetabolites to those purine molecules which are essential for the maintenance of chromosome structure.

BASIC COMPOUNDS

Mutations and chromosome damage have been produced with a number of basic dyes and other bases including acridine orange and pyronine which induced mutations in *Drosophila* (41), pyronine and acriflavine (but not methyl green) which induced mutations in *E. coli* (145), methylene blue and toluidine blue which produced chromosome breaks in onion root tips (16) and putrescine which produced mutations in *Oenothera* (102). These basic substances may be effective by combining with the acid groups of deoxyribonucleic acid and so preventing the

combination with nucleoprotein and the formation of nucleoprotein. These mutagenic bases and the purines do not appear to have been tested for carcinogenic action.

INORGANIC COMPOUNDS

In testing the effects of various substances on *E. coli*, mutations were observed following treatment with ferrous sulphate and manganous chloride without killing many of the organisms (49). Demerec and Hanson (50) used the change from streptomycin dependence of *E. coli* to study the effect of manganous chloride. If the organisms were washed with water or hypotonic solutions of salts or sugar solutions before treatment with manganous chloride the mutagenic effect was decreased, whereas prior washing with hypertonic solutions increased the mutagenic action. Under constant conditions the mutagenic effect is proportional to the concentration of manganous chloride up to 0.005%; it is reduced if sodium chloride is present and completely inhibited by sodium phosphate though not by sodium acetate buffer of the same pH. Many of the conditions which reduce the mutagenic action reduce the uptake of manganese by the cells (122). Under conditions in which copper salts, manganous chloride and ferrous chloride are potent mutagens, the chlorides of cadmium, cerium, cobalt, chromium and mercury, and silver nitrate were ineffective (49).

The nonradioactive metallic salts which appear to have been effectively tested for carcinogenic activity are sodium bisulphite (58), which was non-carcinogenic when given in food to rats and zinc chloride, which induces teratomas when injected into the testis of fowls (15). The salts of manganese, iron and zinc are among those which precipitate nucleoprotein and prevent the swelling of nucleoprotein in low concentrations (2).

Inorganic salts can also have a mutagenic action through the osmotic pressure which they exert. The mutation of the phage B of *E. coli* is increased by hypertonic sodium chloride (96). Hypertonic sodium chloride solutions have induced a few sarcomata in rats (137) and when injected intradermally produce greying of the hair of coloured mice similar to that produced by radiation or nitrogen mustards (31).

EFFECTS OF RADIATION AND MUTAGENS ON NUCLEIC ACID

Chromosomes are very sensitive to the action of radiation and radiomimetic drugs and chromosomes are remarkable for their high concentration of deoxyribonucleic acid. Deoxyribonucleic acid is itself changed when irradiated or treated with some of the radiomimetic agents. Solutions of sodium deoxyribonucleate have a high and anomalous viscosity which is dependent on the ionic strength of the solution, and show streaming birefringence. When such solutions are treated with aliphatic nitrogen mustards a fall in viscosity occurs (62); the fall is greatest in solutions of low ionic strength. As the effect is prevented if thiosulphate is added the action is probably due to reaction of the ethyleneimine transformation products of the nitrogen mustard (because thiosulphate

reacts rapidly with ethyleneimine). A similar change in deoxyribonucleate following X-irradiation was observed by Taylor, Greenstein and Hollaender (135) who noticed that if the irradiation was carried out in the presence of oxygen a slow progressive fall in viscosity occurred in addition to an immediate effect which was independent of the oxygen concentration. The change in viscosity was accompanied by an increase in the degree of dispersion of the material so that the final reaction mixture was polydisperse. The effect was reduced if serum albumen was added to the system.

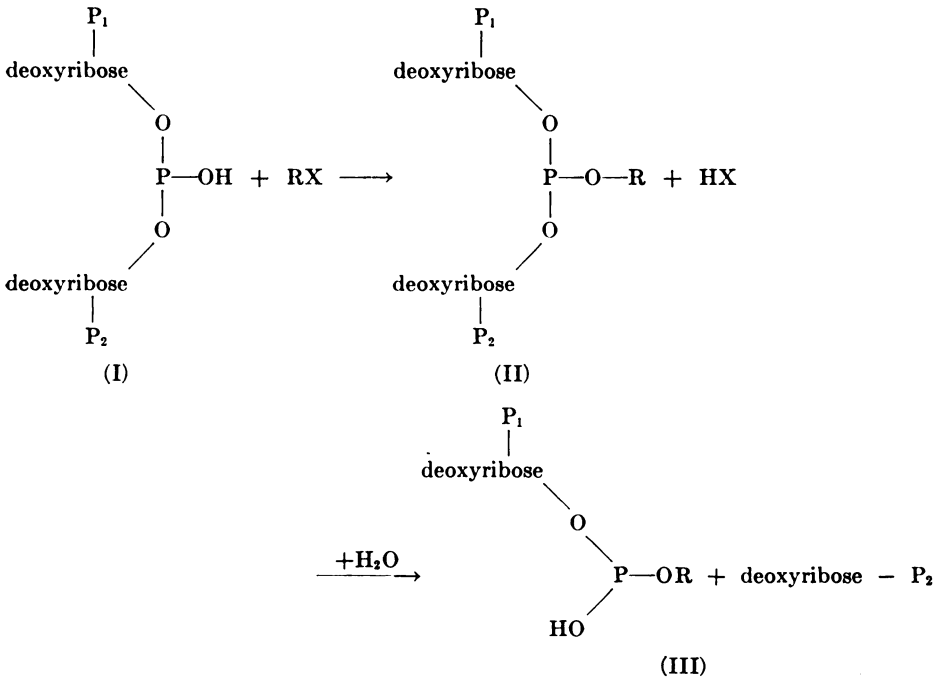
These effects have been studied by Butler and his colleagues (cf. 46) who have also found similar effects with other cytotoxic agents including ethyleneimine, diepoxybutane, triethylene melamine, the monofunctional compound dimethylchloroethylamine ($(CH_3)_2N\ CH_2CH_2Cl$) which is mutagenic in *Neurospora* (94) and semicarbazide (36a) which is mutagenic when fed to *Drosophila* (99, 115). Deoxyribonucleic acid is degraded by free hydroxyl radicals produced chemically (*e.g.*, from hydrogen peroxide and ferrous sulphate) in a similar way to the degradation by irradiation (37). Destruction of deoxyribonucleate occurs *in vivo* as was shown in rats examined 24 hrs. after treatment with nitrogen mustard (40) or after irradiation (97).

The action of radiation on deoxyribonucleic acid is reduced by conditions which reduce the effects of radiation such as cyanide or mercaptoethylamine (13). Many of the facts are in agreement with the possibility that the production of chromosome damage and mutations is due to changes in the deoxyribonucleic acid of the chromosomes. Some other mutagens such as the inorganic metal salts precipitate deoxyribonucleic acid (2) and the carcinogenic hydrocarbons and other polycyclic carcinogens appear to form complexes with purines and deoxyribonucleic acid (25).

The alkylating agents may disrupt the deoxyribonucleic acid chain (I) by esterifying the phosphate group to give a phosphate group esterified to two deoxyribose molecules of the nucleic acid chain and one molecule of the alkylating agent (46). Such a trisubstituted phosphate residue (II) might hydrolyse (45) either to give the original nucleic acid molecule or more probably to give a split nucleic acid with the alkylating agent replacing one part of the original chain (III).

If such a change takes place with the vesicants and other alkylating agents an analogous reaction might occur with peroxide formed by irradiation to give a disubstituted perphosphoric acid derivative (II where R = OH). Such an ester might be less stable than the original phosphate ester and either hydrolyse to yield a split nucleic acid with a perphosphate end group or slowly oxidise the same or another molecule of nucleic acid. Other possible modes of action of alkylating agents, peroxides and radiations include reactions with the amino groups or nitrogen atoms of purines or pyrimidine rings.

The formation of the nucleic acid ester or the peroxide compound may distort the chromosome sufficiently to produce either a mutation or visible chromosome damage. The actual breakdown of the nucleic acid molecule may be a stage of the process which is not essential for the production of the cytogenetical effects.



ANTIMUTAGENS AND ENDOGENOUS MUTAGENS

Some samples of antimutagenic actions have already been mentioned including the suppression of the mutagenic action of manganous chloride on *E. coli* by phosphate buffer or sodium chloride (50) and the effect of thymine in reducing the chromosome damage produced by urethane (30). Another aspect of antimutagenesis consists of photoreactivation of bacteria after exposure to ultraviolet light. If conidia of *Streptomyces griseus* (88) or a T group bacteriophage of *E. coli* (55) are exposed to visible light after ultraviolet light the damaging effect of the ultraviolet irradiation is reduced. The lethal effect of ultraviolet light on *E. coli* can only be partially reversed by visible light (108) suggesting that the damage is of two types. The mutagenic effect of ultraviolet light on *Paramecium* is reversed by visible light (90) but the action of X-rays is not so reversed. Thus under certain conditions visible light has an antimutagenic action.

Novick and Szilard (109) have measured the rate at which mutants of *E. coli* resistant to Phage T5 arose when growing under constant conditions in a chemostat. By this means the "spontaneous" mutation rate under various conditions can be measured. With phosphate, lactate or ammonium chloride as the growth-controlling factor the mutation rates were between 0.53 and 0.82 per 10^8 organisms per hour. With tryptophane or arginine as growth-controlling factors the corresponding rates were between 1.55 and 1.76. When added to this system caffeine, theophylline, theobromine, azaguanine or adenine increased the mutation rates to values which depended on the growth controlling factor used. Later (110) it was found that the mutagenic action of theophylline could be completely

neutralised by guanosine so that guanosine is an antimutagen. Adenosine and inosine were also anti-mutagens for theophylline but xanthine and the free purine, hypoxanthine, had no antimutagenic action.

These results raise the point of the nature of spontaneous mutation. As the mutation rate induced by a mutagen (*e.g.*, theophylline) is dependent on the general nutrition and the presence of antimutagens the "spontaneous" mutation rate is probably also dependent on the ratios of natural constituents of the organisms or media. As the mutation rate is raised threefold by the addition of tryptophane or arginine, these substances must be considered as mutagens although tryptophane has only about a tenth of the activity of theophylline.

TRANSFORMING PRINCIPLES

In 1928 Griffith (67) found a substance present in heat killed encapsulated pneumococci which could induce unencapsulated pneumococci to form capsules. The change was permanent and inherited, and the capsule produced was the same as that of the organism from which the inducing agent was derived. The discoveries of Griffith have been confirmed and extended by Avery (11) who has named the inducing agents "transforming principles." These transforming principles are mutagens with very special properties. First, they are produced by organisms and must be concerned with the maintenance of the properties of the organism. Secondly, they produce specific changes in the organism on which they act, and thirdly, the transformed organisms produce more of the principle. The radiations and chemical mutagens produce random genetical changes, but the transforming principles induce bacteria to synthesise polysaccharides which are specific and distinguishable by chemical and serological reactions.

Encapsulated pneumococci grown on solid media form fluid colonies with smooth surfaces and are of the S type. Such organisms may spontaneously change into rough or R types and lose the power of forming capsules. Although pneumococci change from S to R they never change from one type to another. If organisms of the R type are incubated with transforming principle and agglutinins for the R type in a fluid medium, then encapsulated forms develop in the medium and can be separated by spreading the culture on solid media and examining the colonies which develop.

Chemical investigations have shown that the transforming principles are not polysaccharides, lipoids, proteins or pentose nucleic acids, but are deoxyribonucleic acids. Chemical agents or the enzyme deoxyribonuclease which hydrolyse deoxyribonucleic acid destroy the transforming principles (82). Not only are the transforming principles more specific than chemical mutagens or radiations but they are much more active; a concentration of 1 part in 600,000,000 of the purified pneumococcal transforming principle is effective, so that on a quantitative basis they are about a hundred times as active as nitrogen mustard.

Many transforming principles have now been isolated not only from pneumococci but also from *E. coli* (22) and *H. influenzae* (1, 1a, 36). The principles can induce changes from 1) rough to smooth, 2) rough to "mutant smooth", 3) mutant smooth to normal or other mutant smooth, 4) extreme rough to rough or

the reverse, 5) smooth or rough types with M2 somatic antigenic protein to types with M3 protein (12), 6) normal pneumococci to penicillin resistant (83), 7) normal *Hemophilus influenzae* to streptomycin resistant (1a).

These results show that nucleic acids from different sources have very different biological specificity although they have very similar chemical composition. The effects obtained are in some respects analogous to the effects of viruses but the transforming principles are protein free and resistant to heat and other conditions which denature proteins and inactivate viruses. These transforming principles bear a similar relationship to radiation and chemical mutagens as do tumour viruses to radiation and chemical carcinogens. The transforming principles elicit specific mutations; the chemical mutagens produce almost random mutations. The tumour viruses such as the Rous virus induce one specific type of tumour while one chemical carcinogen may induce many different types of tumour which cannot be predetermined.

CONCLUSIONS

One of the difficulties in the study of mutagenesis is the variability of the results obtained. Although radiations and the active vesicants are active on all systems which have been tested, urethane has no effect when tested on *Neurospora* reversions and ferrous chloride had been found active only when tested on bacteria. This variability makes the comparison with other effects very uncertain. This is particularly the case in the comparison with carcinogenesis, as cancer can be induced only in vertebrate animals and it is difficult, laborious and expensive to measure mutation rates in mammals or other vertebrates.

From the point of view of therapeutics mutagenic action is almost always a disadvantage. If a mutagenic agent is used in the treatment of infectious disease or leucaemia the drug is likely to induce mutant resistant forms. Thus a drug with mutagenic action is more likely to cause drug fastness to develop than is a drug without such action. An example of this is streptomycin which appears to be mutagenic for the tubercle bacillus (103).

The mechanism of mutagenic action which can be brought about by a wide variety of different chemical and physical agents is not known. That such a variety of means should operate through the same mechanism appears to be very unlikely. In the reviewer's opinion the mechanisms by which chromosome breakage, mutagenesis and carcinogenesis are brought about by any one compound are probably similar, being different manifestations of the same effect. A number of possible mechanisms have been suggested for carcinogenic action (27) depending upon different effects on deoxyribonucleic acid of chromosomes and including modification and possibly destruction by irradiation or mustards, precipitation or increased gelation by metal salts, inhibition of synthesis by urethane or antimetabolites and complex formation by polycyclic hydrocarbons and heterocyclic compounds.

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