Mutation Research, 134 (1984) 113–142
Elsevier

MTR 07179

A review of the genetic effects of ethyl methanesulfonate

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(Received 10 October 1983)
(Revision received 23 May 1984)
(Accepted 25 May 1984)

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Summary

Ethyl methanesulfonate (EMS) is a monofunctional ethylating agent that has been found to be mutagenic in a wide variety of genetic test systems from viruses to mammals. It has also been shown to be

* Operated by Martin Marietta Energy Systems, Inc., under contract DE-AC05-840R21400 with the U.S. Department of Energy.

Abbreviations: BMS, butyl methanesulfonate; DEN, diethyl nitrosamine; DMS, dimethyl sulfate; EMS, ethyl methanesulfonate; ENU, *N*-ethyl-*N*-nitrosourea; iPMS, isopropyl methanesulfonate; MMS, methyl methanesulfonate; MNNG, *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine; MNU, *N*-methyl-*N*-nitrosourea; PMS, propyl methanesulfonate.

carcinogenic in mammals. Alkylation of cellular, nucleophilic sites by EMS occurs via a mixed SN_1/SN_2 reaction mechanism. While ethylation of DNA occurs principally at nitrogen positions in the bases, because of the partial SN_1 character of the reaction, EMS is also able to produce significant levels of alkylation at oxygens such as the O^6 of guanine and in the DNA phosphate groups. Genetic data obtained using microorganisms suggest that EMS may produce both GC to AT and AT to GC transition mutations. There is also some evidence that EMS can cause base-pair insertions or deletions as well as more extensive intragenic deletions.

In higher organisms, there is clear-cut evidence that EMS is able to break chromosomes, although the mechanisms involved are not well understood. An often cited hypothesis is that DNA bases ethylated by EMS (mostly the *N*-7 position of guanine) gradually hydrolyze from the deoxyribose on the DNA backbone leaving behind an apurinic (or possibly an apyrimidinic) site that is unstable and can lead to single-strand breakage of the DNA. Data also exist that suggest that ethylation of some chromosomal proteins in mouse spermatids by EMS may be an important factor in causing chromosome breakage.

Introduction

Ethyl methanesulfonate (EMS) was the first agent found unambiguously to increase the proportion of mutants (plaque-type and host-range) in T2 phage treated in vitro with the chemical (Loveless, 1958). Since this early work, EMS has been used in mutational studies in a wide variety of biological test systems. Currently, the Environmental Mutagen Information Center has more than 3400 references on this chemical. In many of these references, however, EMS was only used as a positive control and in a large number of other references EMS was simply used as a means for creating new mutations.

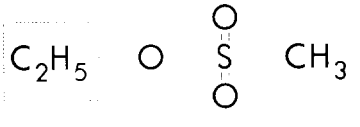
The purpose of this review is to cover broadly the different genetic test systems in which EMS has been used. An attempt has also been made to emphasize those references which provide some insight into the mechanism of action of EMS. Because of the large number of references available on EMS, of necessity, I have been very selective. Readers interested in other papers citing EMS may obtain help from the Environmental Mutagen Information Center in Oak Ridge, Tennessee.

Chemical and physical properties

EMS is a colorless liquid at room temperature. It has a boiling point of 213–213.5 °C (761 mm Hg) and a density of 1.1452 at 22 °C relative to water at 4 °C (IARC, 1974). The molecular weight of the chemical is 124.2 (Sax, 1979) and its index of refraction is 1.4180 at 20 °C (Aldrich, 1982–1983). The structural formula for EMS is shown in Fig. 1.

Synthesis of EMS has been accomplished by reaction of methanesulfonic anhydride with ethyl alcohol (Billeter, 1905). To label the ethyl group of EMS with ^3H or ^{14}C for radio-tracer studies, a favorite method of synthesis has been to react labeled ethyl iodide with silver methanesulfonate (Brookes and Lawley, 1961; Lawley and Brookes, 1963; Swann and Magee, 1971). EMS has been produced for research purposes, but there is no large-scale production of this chemical. It is not known to occur naturally.

EMS owes its biological reactivity to its ethyl group (Fig. 1) that can be transferred to a variety of cellular, nucleophilic sites. For EMS, this transfer of the ethyl group can be accomplished either through an SN_1 (substitution, nucleophilic, unimolecular) or an SN_2 (substitution, nucleophilic, bimolecular) mechanism (Osterman-Golkar et al., 1970; Lawley, 1974a, b). The Swain–Scott sub-



ETHYL METHANESULFONATE
(EMS)
M.W.=124.2

Fig. 1. Structural formula of EMS.

strate constant, s, for EMS has been reported to be 0.64 at 37 °C, increasing to 0.67 at 20 °C (Osterman-Golkar et al., 1970). Generally, chemicals with lower s values tend to react primarily through an SN_1 mechanism, while those with high s values react mostly through an SN_2 mechanism. The s value for EMS is such that it is thought to react through both mechanisms.

Interaction with DNA

Generally, DNA is believed to be the most important target for the induction of mutations by chemical agents and many studies have focused on the interactions between DNA and chemical mutagens. When DNA is treated in vitro or in vivo with chemical mutagens a number of reactive sites have been identified. These include the *N*-7, O^6, *N*-3 and N^2 of guanine; the *N*-1, *N*-3 and *N*-7 of adenine; the O^2, O^4 and *N*-3 of thymine; the O^2 and *N*-3 of cytosine; the phosphate groups on the DNA backbone (Loveless, 1969; Swann and Magee, 1971; Bannon and Verly, 1972; Lawley et al., 1972, 1975; Lawley, 1974a; Singer et al., 1974; Sun and Singer, 1975; Shooter and Merrifield, 1976; Singer, 1976, 1977a, b, 1982; Frei et al., 1978). Not all of these sites have been shown to be ethylated by EMS. However, because EMS can react by a mixed SN_1/SN_2-type mechanism, it is reasonable to assume that all of the above-mentioned sites in DNA can be ethylated to some extent by EMS. Originally, the *N*-7 of guanine was the first site demonstrated to be ethylated by EMS (Brookes and Lawley, 1961). This is also the predominant site of attack by EMS in DNA. As time moved on, more sensitive means of measuring DNA alkylation products were developed. Table 1 lists the current known ethylation products produced in double-stranded DNA after exposure to EMS (Singer, 1982).

Because of the high frequency of 7-alkylguanine residues produced in the reaction of alkylating agents, such as EMS, with DNA, mispairing of this modified purine was once believed to be an important cause of mutations (Auerbach, 1976). Other work has suggested, however, that 7-alkylguanine may not be of great importance in the mechanism of mutagenesis and carcinogenesis of alkylating agents (Swann and Magee, 1971; Lawley, 1974a; Goth and Rajewsky, 1974; Montesano and Bartsch, 1976). For example, guanine alkylated at the *N*-7 position is not likely to be involved in mispairing (Koch and Miller, 1965; Lawley, 1974a). Also, strong mutagenic agents, like EMS and *N*-methyl-*N*-nitrosourea (MNU), and weaker mutagens, such as methyl methanesulfonate (MMS) and dimethyl sulfate (DMS) are both effective in alkylating the *N*-7 position of guanine (Singer, 1982). Loveless (1969) was the first to suggest that mutagenicity may be correlated with the formation of O^6-alkylguanine rather than of *N*-7-alkylguanine. He found evidence for O^6-alkylation by EMS and MNU but not by MMS and DMS. Other workers have also found that chemicals reacting through an SN_1 mechanism produce higher amounts of O^6-alkylguanine relative to *N*-7-alkylguanine than do chemicals reacting by an SN_2-type mechanism (Lawley and Thatcher, 1970; Lawley, 1974a, b). Thus, EMS, which can react by an SN_1 mechanism as well as an SN_2 mechanism, is expected to produce relatively more O^6-ethylation than that found with MMS which reacts via an SN_2-type mechanism.

A number of studies have added support for the

TABLE 1 [a]

ETHYLATION PRODUCTS OF DOUBLE-STRANDED DNA AFTER IN VITRO EXPOSURE TO EMS

Ethylation site	Percent of total DNA ethylation
Guanine	
N-7	65
O^6	2
N-3	0.9
Adenine	
N-3	4.9
N-1	1.7
N-7	1.1
Thymine	
O^2	Not detected
O^4	Not detected
N-3	Not detected
Cytosine	
N-3	0.6
O^2	Not detected
Phosphate	13

[a] Taken from Singer (1982).

involvement of O^6-alkylation in mutagenesis (Gerchman and Ludlum, 1973a, b; Lawley, 1974a; Auerbach, 1976; Newbold et al., 1980; Peterson and Peterson, 1982; Beranek et al., 1983). Gerchman and Ludlum (1973a, b) have presented evidence for the misincorporation of bases by RNA polymerase acting on a template containing O^6-methylguanine. Conversely, similar studies using 7-methylguanine have shown it to have base-pairing properties similar to guanine (Ludlum, 1970; Gerchman and Ludlum, 1973b).

Newbold et al. (1980), using simple aliphatic alkylating agents, found that differences in mutagenicity of the chemicals in V79 Chinese hamster cells closely paralleled differences in levels of O^6-guanine alkylation. Using MNNG and MMS, Peterson and Peterson (1982) found that MNNG produced 12-fold more O^6-methylguanine and 5-fold more mutants in V79 Chinese hamster cells than did MMS. However, neither the cytotoxicities nor the mutagenicities of these methylating agents could be attributed solely to nitrogen or to oxygen methylations in the DNA. Beranek et al. (1983) exposed Chinese hamster ovary cells to various doses of MMS and MNU and then assayed for the induction of 6-thioguanine resistance and for specific DNA adducts. When mutations induced by MMS or MNU were compared by linear correlation analysis with levels of different adducts formed, only O^6-methylguanine displayed a strong correlation with mutations. A similar study was done by Heflich et al. (1982) in which Chinese hamster ovary cells were exposed to different doses of ENU or EMS. Mutation induction was found to be best correlated with the amount of N-3 and O^6-ethylguanine produced.

O^6-Alkylation has also been implicated as an important lesion in carcinogenesis. After exposing neonatal rats to ENU, Goth and Rajewsky (1974) demonstrated that, while N-7-ethylguanine and N-3-ethyladenine were eliminated from brain and liver DNA at similar rates, respectively, O^6-ethylguanine was removed much more slowly from the brain DNA than from the liver DNA. Since the rat nervous system is the primary site of tumor induction by ENU (Druckrey et al., 1970; Swenberg et al., 1972), O^6-ethylguanine may play an important role in the brain tumors produced. While EMS does not produce as high a proportion of ethylation at the O^6 position of guanine as does ENU (Frei et al., 1978; Singer, 1982), it nevertheless does alkylate this site in DNA, and this may be a cause for at least part of the mutagenic activity of EMS.

Alkylation of the O^4 position of thymine is also a likely candidate for mispairing (Lawley, 1974b; Drake and Baltz, 1976). However, ethylation of this site by EMS has not been reported (Singer, 1982). Singer and Fraenkel-Conrat (1969a, b) used MNNG to demonstrate that the amount of alkylation at the N-3 of cytosine in tobacco mosaic virus was correlated with mutagenicity. Since EMS has been shown to alkylate this site to a low extent (Singer, 1982), this may be another source for EMS-induced mutations.

Much less is known about the possible mutagenic effects of alkylation at the N-1 and N-3 positions of guanine and the N-1, N-3 and N-7 positions of adenine except that, in DNA, the N-3- and N-7-alkyl purines have a weakened glycosidic linkage which can lead to the loss of the purine from DNA (Auerbach, 1976; Fishbein et al., 1970; Lawley, 1966, 1974; Lawley and Brookes, 1963; Singer, 1975). Weakening of the glycosidic linkage has also been reported for O^2-alkylthymine (Singer, 1976), and depyrimidination of this alkylated base is likely, although the genetic consequences of this process are unknown.

Evidence has been presented that suggests that depurination of alkylated DNA may be potentially lethal, due to the formation of DNA single-strand breaks (E. Freese, 1971; E. Freese and Freese, 1966; Lawley, 1974b; Lawley and Brookes, 1963; Lawley et al., 1969; Rhaese and Freese, 1969; Strauss and Hill, 1970; Uhlenhopp and Krasna, 1971). It has also been suggested that apurinic sites in DNA might give rise to transversion mutations (Bautz and Freese, 1960; E.B. Freese, 1961). Shearman and Loeb (1977, 1979) and Kunkel et al. (1981), using in vitro techniques as well as ϕX174, have presented evidence indicating that when partially depurinated DNA is used as a template for DNA synthesis, the base inserted opposite the apurinic site appears to be selected randomly. Making use of an amber mutant of ϕX174, Kunkel et al. (1981) found that mutagenesis was enhanced by depurination of the template DNA, presumably as the result of a base substitu-

tion in the amber codon. However, it was not established if transversion mutations were involved.

The possible esterification of phosphodiesters by alkylating agents to form alkyl phosphotriesters in polynucleotides was long suggested, but was only firmly established in the 1970s (Bannon and Verly, 1972; Lawley, 1973; Sun and Singer, 1975; Shooter and Merrifield, 1976). The identification of the alkyl phosphotriester was difficult because it is a major product only when *N*-nitroso compounds are used. Also, the *O*-alkyl group is hydrolyzed under the usual conditions used for hydrolysis of DNA (70% $HClO_4$, 100 °C, 1 h) or RNA (1 N HCl, 100 °C, 1 h) (Singer, 1977b). In general, chemicals with lower *s* values are more efficient in alkylating phosphate groups and other oxygen sites in DNA than are chemicals with higher *s* values (Lawley, 1974b). Bannon and Verly (1972) reported that alkylation of phosphates accounted for 15% of the total DNA alkylation with EMS (*s* value = 0.64) but only 1% by MMS (*s* value = 0.83) Osterman-Golkar et al., 1970).

Phosphate alkylation appears to cause inactivation of RNA in tobacco mosaic virus without causing strand breakage (Singer et al., 1975). It has also been suggested that DNA strand breakage could result from hydrolysis of alkylated phosphate groups (E. Freese, 1971; Singer, 1975; Rhaese and Freese, 1969), which could contribute to chromosome breakage and lethality (E. Freese and Freese, 1966; Rhaese and Boetker, 1973; Singer, 1975, 1976). However, the work by Bannon and Verly (1972), which showed that alkyl phosphotriesters are apparently quite stable, would argue against this model.

Review of mutagenicity data

Effects of EMS in viruses

EMS was discovered by Loveless (1958) to induce mutations in bacteriophage T2 treated in vitro. The effect studied was the conversion of wild-type r^+ (lysis-inhibiting) phage to r (rapid-lysing) mutants that produce a plaque with a clear halo. Green and Krieg (1961) performed similar studies with EMS using the T4 r_{II} system. Exposure to 0.4 M EMS for 60 min inactivated about 50% of the phage. Of the remaining phage, about 1% were r mutants or mottled (plaques composed of a mixture of r and r^+ phage), an increase of 100-fold over the spontaneous mutant frequency. An interesting observation in this work was that every mutant plaque contained both r and r^+ phage particles. The relative proportions of mutants and nonmutants in the mixed clones did not correspond to a simple segregation from phage that contained an extracellularly mutated gene and a nonmutant allele. The frequency distribution of mutants in the mixed clones indicated that a mutant clone could be initiated with an equal probability in any generation of vegetative phage growth.

In a later paper Krieg (1963) concluded that in the T4 r_{II} system, the occurrence of EMS-induced GC to AT transitions was much more common than AT to GC transitions. Krieg suggested that the transitions might be occurring by mispairing with 7-ethylguanine. In light of what is now known about the interaction of EMS with DNA, it is possible that the mixed plaques arose from mispairing with O^6-ethylguanine during different generations of vegetative growth.

Inactivation of T4 phage was studied by Ronen (1968). That work indicated that the immediate inactivation of EMS-treated phage took place as the result of a process other than hydrolysis. It was suggested that the immediate killing of phage resulted from the mere presence of ethyl groups on the phage DNA molecule, while delayed inactivation resulted from the breakdown of the ethylated DNA. Inactivation of T7 phage by EMS was found to follow a similar pattern as that seen for T4 (Verly and Brakier, 1969; Brakier and Verly, 1970; Verly et al., 1974; Karska-Wysocki et al., 1976). Lethality increased with time after the end of the EMS exposure. Delayed inactivation was claimed to be due exclusively to depurination, with one lethal hit corresponding to 7 or 8 depurinations per phage (Brakier and Verly, 1970). 50% of the immediate inactivation of T7 phage was attributed to apurinic sites and single-strand braeks (Verly et al., 1974). Diester phosphate ethylation did not appear to be toxic, while terminal phosphate alkylation may have been. Verly et al. (1974) did not find base ethylation, by itself, to be toxic although mispairing at the transcriptional level may contribute to toxicity. Karska-Wysocki et al.

(1976) found that EMS exposures that inactivated more than 99.5% of the T7 phage had no effect on phage adsorption on *Escherichia coli* B cells but decreased the amount of phage DNA injected into the host cells. Thus, ethylation of phage protein did not appear to be an important mechanism of inactivation. Boulé-Charest and Mamet-Bratley (1972) showed that ethylation of T7 DNA by EMS did not create new initiation sites for *E. coli* RNA polymerase. They concluded that incorrect initiation of RNA synthesis is not a cause of the lethality observed immediately after the end of the EMS treatment.

It was suggested by Brookes and Lawley (1963) that DNA-chain breakage caused by the combined effects of depurination in both strands is the major cause of alkylation-induced inactivation of double-strand phage. However, Ronen (1969) studied λ-phage particles in which one DNA strand was ethylated with EMS while the other was intact and found that there was still delayed inactivation of the λ phage. He concluded that events taking place in only one DNA strand are sufficient to bring about inactivation.

Boyce and Farley (1968) studied the production of single-strand breaks in covalent circular λ phage exposed to EMS. They found that breaks were induced only in the presence of a heat-labile factor from extracts of *E. coli*. These data suggested that enzymatic mechanisms were involved in the production of single-strand breaks. The rate of formation of the 'enzyme-sensitive sites' with EMS was found to be about 20 times slower than with MMS.

Modified DNA polymerases acting upon alkylated DNA templates may also play a role in mutation induction. For example, Drake and Greening (1970) showed that an antimutagenic DNA polymerase of phage T4 was able to moderately suppress the mutagenic activity of EMS at G : C sites within the r_{II} locus.

Genetic effects in prokaryotes

The high mutagenic activity of EMS has been demonstrated in a wide variety of bacterial genera, species and strains including *Escherichia coli* (Kondo et al., 1970; Hill, 1972; Schwartz, 1963; B.S. Strauss, 1962; Howell-Saxton et al., 1973; Mohn et al., 1974; Ishii and Kondo, 1975; Nestmann, 1975; Green et al., 1977), *Salmonella typhimurium* (McCann et al., 1975a, b; Skopek et al., 1978; McMahon et al., 1979), *Proteus mirabilis* (Böhme, 1968; Adler et al., 1976), *Haemophilus influenzae* (Kimball and Hirsch, 1975; Notani et al., 1975), *Staphylococcus aureus* (Van der Vijver et al., 1975), Mycobacterium (Koníčková-Radochová and Málek, 1969; MacNaughton and Winder, 1977; Kolman and Ehrenberg, 1978), *Micrococcus glutamicus* (Shanthamma et al., 1972), *Rhizobium trifolii* (Kaushik and Venkataraman, 1972), *Pseudomonas aeruginosa* (Chandler and Krishnapillai, 1974), and *Dictyostelium discoideum* (slime molds) (Payez and Deering, 1972). EMS has also been shown to be mutagenic in *Micrococcus radiodurans* (Sweet and Moseley, 1976) although considerably less so than in *E. coli*.

The most widely used bacterial system for the detection of mutations is that developed by Ames and coworkers (Ames, 1971; Ames et al., 1973, 1975; McCann et al., 1975a, b) using *Salmonella typhimurium*. The test is based on the reversion of histidine-requiring mutants. A variety of strains have been developed that can be used to test for base-substitution or frameshift mutations. McCann et al. (1975a) demonstrated that 5000 μg of EMS per plate gave significant increases in revertant colonies using TA1535 and TA100 strains, which detect base-substitution mutations. However, strains TA1538 and TA98, which detect frameshift mutations, showed very few revertants. When a comparison is made with the spontaneous revertants found in these latter two strains, it is doubtful that EMS was, in fact, inducing any frameshift mutations. Mitchell (1974) reported EMS induced a high frequency of revertants in *S. typhimurium* strain *his*G46, which detected base substitutions, but did not induce revertants in strain TA1532, TA1534 or D3052, which detect frameshift mutations. This same author also showed that EMS was effective in reverting *trp*$^-$ (trytophan-requiring) mutants of *E. coli* WP2, which pick up transition mutations.

Anderson and Styles (1978) using an S9 mix from livers of Aroclor-induced animals observed EMS-induced revertants with Salmonella strains TA1538, TA98 and TA1537, that detect frameshift mutations. However, McCann et al. (1975a) and Brusick and Zeiger (1972), without using an S9

mix, did not find EMS-induced revertants of these strains. The mechanism by which the S9 mix could have metabolized EMS to cause it to revert frameshift mutations is unclear.

Verly et al. (1968) demonstrated that single-strand breaks in *E. coli* DNA induced by EMS can be repaired during a latency period. Skjåk Braek and Smith-Kielland (1974) found that DNA repair synthesis occurs in *E. coli* after exposure to EMS, while Billen and Hellerman (1977) showed that EMS is a poor inducer of DNA polymerase I repair activity in permeabilized *E. coli*.

In repair-deficient pol$^-$ A and rec$^-$ A strains of *E. coli*, it was found (Howell-Saxton et al., 1973) that logarithmic-phase cells of both mutants and the parental strains exhibited a greater sensitivity to EMS than the stationary-phase cells. However, the pol$^-$ A and rec$^-$ A mutants were 3–4 times more susceptible to inactivation by EMS than were the parental strains.

Fluck et al. (1976) demonstrated that the zone of growth inhibition around a center well of EMS was 50% greater with pol$^-$ mutants than with pol$^+$. Rec$^-$ cells are less able than wild-type to reactivate phage λ damaged by EMS alkylations (Ronen and Atidia, 1971) and are more mutable with EMS than are wild-type cells (Böhme and Geissler, 1968). Also, Howell-Saxton et al. (1973) found that both repair-deficient strains exhibited significantly higher EMS-induced mutation frequencies in comparison with the parental strains. In a later paper (Howell-Saxton et al., 1974), it was demonstrated that the alkylated DNA in the mutants contained a greater proportion of small DNA fragments compared with the parental strains.

EMS, unlike MNNG, does not produce linked mutations (Guerola et al., 1971; Guerola and Cerdá-Olmedo, 1975) and does not seem to act at the replicating fork (Nestmann, 1975). In addition, EMS does not appear able to induce any of the SOS functions (Radman et al., 1977; Moreau and Devoret, 1977).

An interesting study by Jeggo et al. (1977) showed that when *E. coli* was exposed to a low adaptive exposure of MNNG (1 μg/ml for 90 min), mutagenesis from subsequent exposures to EMS was significantly reduced. Similarly, low adaptive exposures to EMS resulted in reduced cell killing and mutagenesis by MNNG. The results suggested that the adapative response could be elicited by, and act upon, EMS-derived lesions even though these lesions do not appear to be preferentially located at the replicative fork.

Karran et al. (1979) were able to show that after an adaptive exposure of *E. coli* to MNNG, the crude cell extract was able to remove O^6-methylguanine residues from DNA while crude extracts from non-adapted cells lacked this activity. Subsequent work by Olsson and Lindahl (1980) showed that removal of the methyl group from the O^6 position of guanine is accomplished through an O^6-alkylguanine acceptor protein. The methyl group is transferred from the O^6 position of guanine to a cysteine residue in the protein, thereby inactivating the protein. Sedgwick and Lindahl (1982) found that the same protein was effective in removing O^6-ethylguanine lesions from DNA. The results reported by Jeggo et al. (1977) may, therefore, have resulted from repair of O^6-ethylguanine lesions produced in the *E. coli* DNA by EMS.

Genetic effects in fungi

In *Schizosaccharomyces pombe*, Loprieno (1966) and Loprieno et al. (1969) found that EMS induced almost exclusively (95–99%) base-pair substitution mutations. They observed that for comparable lethality, EMS induced a higher mutation frequency at the ade_6 and ade_7 loci of *S. pombe* than did MMS. Also, EMS induced a percentage of mosaic mutations that was twice that induced by MMS (Loprieno, 1966). Leaky mutants occurred more frequently after EMS treatment (48%) than after MMS treatment (22%). Loprieno (1966) suggested that the leaky mutants were mostly the result of a slightly modified enzyme whose activity was not drastically different from the wild-type enzyme. de Serres (1964, 1966) considered the leaky mutants occurring at the *ad-3A* and *-3B* loci of *Neurospora crassa* to be an indication of missense mutations. Since EMS induced more leaky mutants than MMS in *S. pombe*, Loprieno (1966) concluded that base-pair substitutions giving missense mutations arise more frequently with EMS than with MMS.

Malling and de Serres (1968) analyzed 76 EMS-induced *ad-3B* mutants in *N. crassa* for their reversion response. They concluded that 41% of

the mutants involved a GC to AT base-pair transition, while 17% were AT to GC transition mutations. In addition, 9% of the mutants appeared to involve a base-pair insertion or deletion and 7% were nonrevertible. They suggested that the nonrevertible mutants involved more extensive intragenic deletions.

Most of the mutational studies with EMS in a fungal test system have been carried out with the yeast *Saccharomyces cerevisiae*. Lemontt (1972) studied 3 *rev* mutants in *S. cerevisiae* which reduced ultraviolet (UV) light-induced reversion frequencies at the *ade-1* and *ade-2* loci. These *rev* strains also showed reduced forward-mutation frequencies with UV light. However, when the same *rev* mutants were exposed to EMS, there was little effect on the forward-mutation frequencies induced. There was about a 50% reduction in the reversion-mutation frequencies induced by EMS in the *rev* mutants, but this was considered as probably not significant. The work indicated that UV-induced lesions in the DNA of *S. cerevisiae* were repaired through different mechanisms than those used for repairing EMS-induced lesions in DNA.

X-ray sensitive (*rad*) mutants of *S. cerevisiae* were studied by Evans and Parry (1972). Five *rad* mutants sensitive to X-ray killing were also sensitive to killing by EMS as well as by UV light and heat. Another mutant, at the *rad-3* locus, was isolated on the basis of its primary sensitivity to UV and showed sensitivity to UV alone. The authors concluded that there are at least two separable pathways of repair present in *S. cerevisiae*, one effective in the repair of UV damage and the other effective in the repair of alkylating agents like EMS, ionizing radiation, heat and a fraction of UV damage. Waters and Parry (1973) studied a number of *rad-3* mutants which all showed increased sensitivity to the lethal effects of UV light. In contrast to UV, however, EMS treatment produced only small differences in the sensitivities of the *rad-3* cultures compared with wild-type cultures. Assuming that the lethal effects of EMS result from DNA single-strand breaks, the results suggested that the product of the *rad-3* gene is involved in the repair of UV-induced pyrimidine dimers but not in the repair of EMS-induced single-strand breaks.

Prakash and Sherman (1973) and Prakash (1974, 1976) studied the effects of EMS on the *cy-1* locus in *S. cerevisiae*. This locus involves the structural gene determining iso-1-cytochrome *c*. Through reversion analysis of a number of mutants at this locus, it was found that EMS appeared selective in producing GC to AT base-pair transitions, while MMS showed no apparent specificity (Prakash and Sherman, 1973). Prakash (1974, 1976) combined *cy-1* mutants with a number of *rad* mutants and studied EMS-induced reversion frequencies at the *cy-1* locus. All of the *rad* mutants studied conferred greater UV-radiation sensitivity to *S. cerevisiae* and all of the *rad* mutants showed a greatly decreased reversion frequency at the *cy-1* locus after exposure to EMS. Although the nature of the repair pathways may differ for UV-induced and chemically induced mutations in yeast, a functional repair system appears to be required for the induction of mutations by chemicals like EMS.

McGregor (1974) found that EMS, in the presence of rat liver microsomes, produced a significantly higher gene conversion frequency at the *ade-2* locus in *S. cerevisiae* than it did in the absence of the microsomes. Gene conversion involves a mechanism by which small regions of DNA in a diploid genetic structure are made homozygous. From the results of McGregor's (1974) studies, he concluded that the liver microsomes were enhancing gene conversion induction by EMS through a mechanism not involving activation of the alkylating agent. One suggestion made was that the microsomes might have caused an increased membrane permeability to the EMS.

Loprieno et al. (1974) found that the frequency of EMS-induced gene mutations in the haploid *Schizosaccharomyces pombe* were the same whether in the presence or absence of mouse liver microsomes. However, when gene conversion was studied at the *ade-2* and *trp-5* locus in diploid *S. cerevisiae*, they found that EMS was significantly more effective in causing gene conversions when mouse liver microsomes were present. The kinetics of inactivation of the cells by EMS was a double-hit process both in the presence and absence of the microsomes. The kinetics of gene conversion by EMS at the *trp-5* locus appeared to be a 2-hit process while at the *ade-2* locus it was a 1-hit process, but the same kinetics were maintained in the presence of the microsomes. This led the authors to conclude

that the microsomal system was somehow increasing the spontaneous alkylation reaction.

It has been suggested, mainly from studies done with X-rays (Manney and Mortimer, 1964), that the mechanism for gene conversion depends on the production of single-strand breaks. X-ray treatment produces gene conversion with 1-hit kinetics. Snow and Korch (1970) suggested that the basis for the 1-hit kinetics with X-rays should depend on the breakage of two strands by a single X-ray, while 2-hit kinetics for alkylating agents, such as EMS, should be the result of two independent alkylation events occurring on the two different strands. However, this still leaves unexplained the 1-hit kinetic pattern induced by EMS for gene conversion at the *ade-2* locus in *S. cerevisiae*, noted by Loprieno et al. (1974).

Brusick and Andrews (1974) studied the effects of EMS in 3 strains of *S. cerevisiae*, D_3, D_4 and D_5. Strains D_3 and D_4 were designed to detect mitotic recombination and mitotic gene conversion, respectively, whereas, strain D_5 was designed to detect mitotic recombination and probably mitotic gene conversion events simultaneously (Zimmerman et al., 1966; Zimmerman and Schwaier, 1967; Zimmermann, 1973). EMS appeared to be most toxic in strain D_3. Strain D_5 also demonstrated a strong response to EMS. Good dose–response effects were observed in both the D_3 and D_5 strains. However, strain D_4 did not appear to be as susceptible to either the genetic or toxic effects of EMS.

Davies and Parry (1976) showed that stationary-phase cultures of *S. cerevisiae* were more resistant to the toxic effects of EMS than were cultures in the growth phase. At the same time, the stationary cultures showed a higher gene conversion frequency than did the growing cultures. The investigators concluded that the results were consisted with their hypothesis that the stationary-phase cells were more active in postreplication repair. Wilmore and Parry (1976) demonstrated that after EMS treatment of *S. cerevisiae*, there was a division delay of the cells when inoculated into fresh medium and, when cell division started again, there was degradation of up to 20% of the nuclear DNA. The period of DNA degradation correlated closely with the time at which yeast cultures undergo mitotic recombination and appears to represent the degradation of DNA during a postreplication repair process.

Genetic effects in plants

There is voluminous literature dealing with the effects of EMS in different plant systems. A large number of these papers deal primarily with the creation of new mutant varieties. The papers cited here are those which I feel contribute some information on how EMS interacts with the plant systems.

Some plants in which EMS mutagenesis has been studied include barley (Minocha and Arnason, 1962; Arnason et al., 1962; Froese-Gertzen et al., 1964; Gichner and Omura, 1971, 1972; Gichner et al., 1977; Khalatkar et al., 1977), rice (Nerkar et al., 1970; Rahman and Soriano, 1972; Yamaguchi and Matsubayashi, 1973; Siddiq, 1974; Jana and Roy, 1975; Augustine et al., 1975; Bhan and Kaul, 1976; Rao, 1977), corn (Neuffer and Ficsor, 1963; Efron, 1974; Regiroli and Gavazzi, 1975; Conger and Carabia, 1977), sorghum (Ramulu, 1971, 1974), wheat (Bari, 1963), tomato (Butani et al., 1972; Chakrawarti and Sharma, 1973), pea (Narsinghani and Kumar, 1971, 1974; Panosyan and Tamrazyan, 1973; Hung and Nagy-Porpáczy, 1974), Tradescantia (Sparrow et al., 1974; Nauman et al., 1976; Ichikawa and Takahashi, 1978) and *Vicia faba* (Natarajan and Upadhya, 1964; Swietlińska and Žuk, 1974; Sturelid and Kihlman, 1978; Kihlman and Sturelid, 1978).

Froese-Gertzen et al. (1964) reported that EMS treatment resulted in a pronounced decrease in fertility in barley, although it had little effect on seedling growth and survival. The frequency of chromosome aberrations induced by EMS was low. Cytological analyses of meiotic as well as of mitotic cells treated with the highest concentration of EMS (0.12 M for 1.75 h) exhibited less than one chromosome aberration per cell. These results confirmed those reported earlier by Konzak et al. (1961) as well as the findings of Rieger and Michaelis (1960) with *Vicia faba*. EMS also produced a high frequency of chlorophyll-deficient mutants in both the M_1 and M_2 generation (Froese-Gertzen et al., 1964). Also the spectra of barley mutations induced by X-rays and EMS appear to be different (Swaminathan et al., 1962; Ehrenberg et al., 1961).

In experiments with partially hydrolyzed EMS solutions, Froese-Gertzen et al. (1964) observed a decrease in seedling growth and plant survival, whereas, fertility was less affected. As expected, the mutation rates decreased with increasing hydrolysis of the EMS solutions. The EMS hydrolysis products lowered the pH of the solutions in which the barley was treated, and the workers concluded that high hydrogen ion concentrations might be affecting growth by inhibition of metabolic activity or enzyme inactivation.

A number of papers studying the action of EMS on barley have considered the effects of treatment and storage conditions. Gichner and Omura (1971) reported that after EMS exposure of barley seeds, storage at 25 °C and with 11–20% moisture content lead to a considerable increase in the toxic and genetic effects. These effects did not depend on the pH of the EMS solutions or on the method of washing the seeds before storage (Gichner and Omura, 1972). With a decrease in the EMS dose, the storage effect was delayed.

When EMS-treated barley seeds were stored with a higher water content (30%) for 1–4 weeks, Gichner et al. (1977) found recovery from mutagen-induced injury. The level and speed of recovery depended on the EMS dose and storage period. Seeds treated with very high EMS doses that reduced the level of germination to 5–10% could not recover from the induced injury.

The 30% water content in the seeds allows for metabolic activity (Švachulová et al., 1973) but the seeds do not germinate; if any DNA replication takes place, its rate is very low (Fousová et al., 1974). These conditions in the seeds favor repair processes, as was demonstrated by the repair of single-strand breaks in alkylated DNA from barley seeds stored at 30% water content (Velemínský et al., 1972).

Storage of EMS-treated barley seeds with 20% water content led to an increase in genetic damage (Gichner et al., 1977). The decreased water content in the seeds reduces the aerobic respiration to low levels (Švachulová et al., 1973) and slows recovery capacity (Gichner and Velemínský, 1972). The increase in genetic damage was accompanied by an enhancement of the amount of single-strand breaks resulting from depurination and backbone breakage of DNA (Velemínský et al., 1973).

Khalatkar et al. (1977), using ^{3}H-labeled EMS, found that enhanced mutation frequencies of barley in the M_2 generation, when presoaked seeds were treated, was partly due to greater uptake of the EMS. Drying of seeds after treatment with the EMS and storing for 7 days increased alkylation of the trichloroacetic acid-insoluble fraction and enhanced the mutagenic effects. They concluded that at least some of the differences in mutation frequencies after various treatment procedures were due to variation in the actual dose of EMS ultimately received by the target cells.

With rice, EMS is an effective mutagen only in dehusked seeds (Nerkar et al., 1970; Rahman and Soriano, 1972). EMS apparently does not readily penetrate the water-resistant corky layer of the bran. Nerkar et al. (1970) found that with increased pressures (up to 20 atmospheres) EMS was more effective in altering rice seedling growth, presumably because of enhanced permeability of cell membranes which facilitated the entry of the EMS.

Rahman and Soriano (1972) found that with EMS exposures that did not affect rice germination and survival, there was a reduction in seedling growth and M_1 panicle fertility. As the EMS exposures increased there was an exponential increase in the frequency of chlorophyll mutations. Yamaguchi and Matsubayashi (1973) were able to study the effects of EMS in rice in various stages of the cell cycle by soaking rice seeds in water for various time periods prior to EMS treatment. They observed that mutation frequency was very high in late G_1, decreased suddenly at middle S, increased strongly at late S–early G_2 and thereafter decreased. After EMS treatment of rice seeds, Siddiq (1974) analyzed pollen mother cells at diakinesis and metaphase and found an increasing number of chromosome breaks with increasing EMS exposure.

Bhan and Kaul (1976) found that chlorophyll mutation frequencies in rice were enhanced with increased exposures of seeds to EMS. At very high EMS exposures ($> 90\%$ seedling lethality in the M_1), there was a drop in the mutation frequency. The investigators attributed this to either cell selection or to a high resistance of some seeds to mutagenesis. They also obtained a much greater proportion of albinas with EMS than they did with γ-rays.

In corn, Neuffer and Ficsor (1963) used 3 closely linked genetic markers to show that EMS treatment of corn pollen resulted in measurable losses of the markers. The simultaneous loss of the markers was taken as evidence of chromosomal breakage. Regiroli and Gavazzi (1975) studied the R locus (a complementary factor required for anthocyanin synthesis) in corn. EMS treatment of seeds homozygous for various R combinations induced mutant R sectors on M_1 ears. EMS also induced significant increases in mutations affecting endosperm morphology and chlorophyll synthesis. Conger and Carabia (1977) found that EMS treatment of dormant or presoaked corn seeds both produced mutants at the yg_2 (yellow-green) locus, but the EMS was more effective and efficient in the presoaked seeds.

Ramulu (1971) found that the chiasma frequency in sorghum was reduced after exposure of seeds to EMS. It was suggested that the decrease in chiasma frequency might be due to a disturbance in chromosome coiling, restricted pairing at pachytene or delay in DNA synthesis. EMS was also shown to cause an increase in the frequency of M_2 chlorophyll mutations in sorghum after exposure of the seeds. A greater mutagenic effect was seen when the seeds were soaked before EMS treatment (Ramulu, 1974).

The effect of EMS alone and in combination with copper was studied in wheat by Bari (1963). It was found that seeds exposed to a 1.5% solution of EMS resulted in a 20% frequency of chromosome breaks in root tips of the seedlings. When the seeds were exposed to the EMS in the presence of 10^{-3} mM copper, the frequency of breaks was nearly doubled. Since copper appeared to stimulate seedling growth, it was concluded that EMS was more effective in inducing chromosome aberrations when growth was stimulated.

Using the pea, Narsinghani and Kumar (1971) found that gibberellic acid administered to pea seeds immediately after EMS exposure reduced the chromosome aberrations present at meiotic metaphase in the floral buds. As suggested by Varner (1964) increased protein synthesis stimulated by gibberellic acid may aid in the repair mechanism. Panosyan and Tamrazyan (1973) found that when histones were added to EMS solutions in which pea seeds were soaking, the inhibitory effect of EMS on plant emergence could be completely reversed. Presumably the nucleophilic sites in the histones were absorbing much of the EMS, thereby reducing the EMS alkylation of the pea seeds.

Using Tradescantia, Sparrow et al. (1974) were able to demonstrate mutagenesis of EMS vapor in stamen hairs of exposed inflorescences. The experimenters found that as the EMS concentration increased from 5 to 45 parts per million there was a concomitant increase in the frequency of mutant pink or colorless cells in the stamen hairs. In a later paper, Nauman et al. (1976) reported that exposure–response curves for EMS showed an exponential rise in stamen hair mutations followed by an area of saturation with increasing EMS exposures. The same exposure–response pattern seen with EMS was also observed with X-rays.

After exposing secondary root meristems of *Vicia faba* to a 0.2% aqueous solution of EMS for 2 h, Natarajan and Upadhya (1964) screened for chromosome breaks in metaphase and anaphase cells 12–84 h after the exposures. The breakage points were found to be localized in heterochromatic regions. Also, centromeric regions were affected by EMS, as revealed by centric breaks and inactivation of centromeric activity. Swietlińska and Žuk (1974) found that induction of chromosome aberrations by EMS in Vicia root tips was highly potentiated by caffeine post-treatment. It was at first thought that caffeine exerted its enhancing effects through inhibition of protein synthesis, but cycloheximide, another inhibitor of protein synthesis, did not enhance the yield of chromosome aberrations induced by EMS. Kihlman and Sturelid (1978) reported that in addition to inducing chromosome aberrations in Vicia, EMS strongly increased the frequency of sister-chromatid exchanges (SCEs). While caffeine strongly increased the frequency of chromosome aberrations induced by EMS, it had no significant effect on the frequency of EMS-induced SCEs. This suggested that the mechanisms by which EMS induces chromosome aberrations and SCEs are at least partly different.

Insects

Drosophila

By far, most of the mutagenicity studies of

EMS in insects have been done using *Drosophila melanogaster*. EMS is a very potent mutagen in Drosophila, inducing recessive lethal mutations (Fahmy and Fahmy, 1961; Alderson, 1965; Epler, 1966; Lim and Snyder, 1968; Lewis and Bacher, 1968; Hotchkiss and Lim, 1968; Browning, 1970; Watson, 1972; Lee, 1976; Aaron and Lee, 1977), specific locus mutations (Jenkins, 1967a, b, 1969; Lee et al., 1970; Lim and Snyder, 1974; Huang and Baker, 1976), translocations (Lim and Snyder, 1968; Hotchkiss and Lim, 1968; Watson, 1972; Abrahamson and Lewis, 1971), dominant lethals (Srám, 1970; Lee, 1976), and partial and complete chromosome loss (Bishop and Lee, 1969; Lee, 1976; Schalet, 1977).

EMS acts primarily on postmeiotic stages. Meiotic and premeiotic stages are relatively immune to its effects. Browning (1970) reported a 4–5-fold higher yield of recessive lethals in postmeiotic cells compared with the yield in meiotic and premeiotic cells. Fahmy and Fahmy (1961) reported more than an order of magnitude difference in recessive lethals produced in postmeiotic stages as compared to premeiotic stages. A similarly low mutagenic response of earlier germ cell stages to EMS was reported by Jenkins (1967a, b, 1969). He found that 90% of all EMS-induced *dumpy* mutations occurred in broods from postmeiotic germ cells. All of these observations were consistent with the findings of Aaron et al. (1973) that retained levels of DNA ethylation were much higher in germ cells treated as mature sperm and late spermatids than in those treated as early spermatids or spermatogonia.

Besides producing 'complete' mutants or recessive lethals in Drosophila, EMS is also able to produce 'mosaics', in which the F_1 progeny from treated male parents may contain both mutant and nonmutant cells. In the case of sex-linked recessive lethals, for example, it is possible to detect F_1 females which are germinally mosaic for a lethal by sampling several heterozygous F_2 virgin females from a non-lethal culture, and mating these to males which have inversions on their X chromosome to prevent crossing-over with the X chromosome under test. Since each F_2 female receives only one paternal X chromosome from the F_1 female (which can be either normal or lethally mutated when it arises from an F_1 female germinally mosaic for a lethal), F_1 lethal mosaicism is detected by the presence of both non-lethal and lethal cultures in the F_3 sample from a non-lethal F_2 culture.

Both Alderson (1965) and Epler (1966) found the frequency of complete F_2-sex-linked lethals to be greater than the frequency of F_3 mosaic sex-linked lethals. Epler (1966) also found that the frequency of complete sex-linked lethals induced by EMS increased in a linear fashion with exposure, while the frequency of mosaic sex-linked lethals increased at the lower concentrations of EMS but decreased at the higher concentrations. However, Jenkins (1967b) using the autosomal visible mutant *dumpy*, found a difference in the frequencies of mosaic and complete mutations which was the reverse of that reported by Epler (1966). As pointed out by Jenkins (1967b) the difference may have resulted from the fact that the sex-linked lethal test involves an entire chromosome and would produce a much greater number of double mosaic mutants than would a test involving a single gene. (A double mosaic is defined as two independently induced mutations at different sites along the two DNA strands of a postmeiotic chromosome with one mutation in each strand.) These double mosaic sex-linked lethals woul be classified as complete mutations, and their frequency would increase with exposure.

Aaron and Lee (1977) found that an EMS concentration of 0.1 mM fed to Drosophila males over a 24-h period ethylated sperm DNA to the extent of 2.1×10^{-4} ethylations per deoxynucleotide (dN). This was the lowest EMS exposure that produced an adverse effect in Drosophila, producing a sex-linked recessive lethal frequency of 0.55%. The frequency of these recessive lethals was found to be linear over a 60-fold range in the exposure. Lee (1976) suggested that in those experiments with EMS where exposure–effect relationships were determined for recessive lethals and another type of genetic damage, the recessive-lethal frequencies could serve as a secondary dosimeter to establish dose (ethylations/dN). Thus, for example, the linear relationship that Huang and Baker (1976) found between EMS-induced recessive lethals and *Minute* mutants implies a linear relationship must also exist between dose and *Minute* mutations.

Most of the genetic effects produced by EMS in Drosophila appear to arise from intralocus mutations. Bishop and Lee (1969) studied 83 mutations on the X chromosome at the *yellow* and *white* locus and found all to be intragenic changes. Hochman (1971) found that 74 out of 75 lethals on chromosome 4 affected only a single complementation unit. Lim and Snyder (1974) found that all 83 EMS-induced lethals in the *zeste-white* region of the X chromosome affected only a single complementation unit.

A few EMS-induced lethals affecting more than one complementation group have been observed (Lifschytz and Falk, 1969; Schalet and Lefevre, 1973; Wright et al., 1976). Some of these have been confirmed cytologically as interstitial deletions involving the loss of several salivary chromosome bands (Wright et al., 1976). In general, however, deletions and gross structural rearrangements are rare among EMS-induced recessive-lethal mutations.

The lowest EMS concentration reported to induce translocations in Drosophila is 10 mM. When injected, this EMS concentration produces a 0.2% translocation frequency (Lim and Snyder, 1968, 1973) and when fed for 18 h the frequency was 0.38% (Watson, 1972). A higher translocation frequency of 2.5% was observed after feeding adult males for 24 h with a 25 mM EMS solution (Hotchkiss and Lim, 1968). This same treatment resulted in a 54% sex-linked recessive-lethal frequency. EMS-induced dominant lethals in Drosophila are also relatively rare unless the exposures are very high. Srám (1970) was able to produce a 60% dominant-lethal frequency in Drosophila, but this required the injection of a 50 mM EMS solution.

Drosophila females are capable of storing viable sperm for periods of at least 1–2 weeks. Storage can also be enhanced by maintaining the females on sugar agar, which inhibits egg laying and slows the rate at which the sperm are used in fertilization. After storage of EMS-treated Drosophila sperm, Khan (1969) found no significant reduction in the frequency of F_1 germinally complete and F_1 germinally mosaic sex-linked recessive-lethal mutations. However, Srám (1970), using dominant lethals, Abrahamson and Lewis (1971), using translocations, and Schalet (1977), using sex-chromosome marker loss, found increased genetic damage after storage.

Vogel and Natarajan (1979a, b) studied the mutagenic activity of a number of chemical agents, including EMS, in Drosophila. They found that, in general, agents with the lowest Swain–Scott *s* values (ENU and DEN) did not produce translocations although they did produce recessive lethals. The highest chromosome-breaking activity (translocations and sex chromosome loss) was found with agents having high *s* values (MMS and DMS). EMS was found to be somewhat unique, in that it was slightly less effective in the translocation test, and also less cytotoxic but more mutagenic in the recessive-lethal test than what would have been expected from its *s* value. The authors took this as an indication of the influence on biological effectiveness of factors other than *s* value, such as methylation versus ethylation and the lipid/water partition ratio. The over-all conclusion of these workers was that two factors were very important in determining the genetic damage produced by alkylating agents in Drosophila. These two factors were dose (intensity of alkylation) and reaction pattern (site of alkylation).

Other insects

Injections of EMS into adult house flies (*Musca domestica*) by La Chance et al. (1969) produced dominant lethals in mature sperm. An injection of 20 μg of EMS resulted in nearly 100% lethality. However, 2 weeks after the EMS treatment, spermatogenic activity in the testes had returned to near normal levels. McDonald and Overland (1974) also were able to recover some dominant, heat-sensitive lethal mutations after treatment of house fly sperm with EMS. However, they were relatively rare events, with only 2 stable mutants recovered from among 18 000 chromosomes tested.

La Chance and Leverich (1969) fed EMS solutions containing 10% sucrose to Habrobracon males. Even starved males would not drink solutions containing 2.5% or more EMS, but after drinking 0.5 and 1.0% solutions of EMS the wasps were completely sterilized. Hatchability in the treated groups was considerably higher than expected if only dominant lethals had been induced. (In Habrobracon, females normally fertilize ~ 66% of their eggs, which produce daughters; the un-

fertilized eggs produce haploid sons. Total sterility of the treated males is indicated by the complete absence of daughters among the progeny. If sterility is the result of dominant-lethal mutations, then hatchability of the eggs will be ~ 34%, and all progeny will be haploid males. When sterility is the result of total sperm inactivation, no eggs are fertilized and all hatch to produce haploid males.) On the basis of hatchability, it was estimated that 9–18% of the sperm were inactivated, and the remainder of the sterility was apparently caused by dominant-lethal mutations in the sperm.

Using Habrobracon females, Löbbecke and von Borstel (1962) found that after aerosol exposures to EMS, dominant lethals were induced in oocytes in first meiotic metaphase and prophase. However, the metaphase oocytes were about 20 times more sensitive than those in prophase. The same pattern of dominant-lethal sensitivity was seen with X-rays. When recessive lethality was used as the criterion of genetic damage, EMS affected first meiotic metaphase and prophase oocytes to the same extent. The metaphase oocytes were, however, still 20 times more sensitive to the induction of recessive lethals by X-rays than were the prophase oocytes.

Hoffman and Grosch (1971) also using Habrobracon females, found that there was a dose-related reduction in hatchability and egg production when EMS was fed or injected, but not when it was applied topically or as an aerosol. Also fewer eggs were deposited and more embryonic deaths resulted during cleavage when starved wasps were injected. The authors suggested that the EMS dose to the germ cells may have been higher in the starved insects since the fat bodies, which would normally absorb part of the EMS dose, were greatly reduced in the starved wasps.

Sensitivity of male germ cells in the mulberry silkworm, *Bombyx mori*, to EMS was studied by Datta et al. (1978). Larvae and pupae were injected with 0.05, 0.1 and 0.15% solutions of EMS. Dominant lethals were induced in spermatocytes, spermatids and spermatozoan stages, with the latter two stages being most sensitive. Cytological studies of metaphase I spermatocytes showed a variety of chromosome aberrations induced by EMS, including aneuploidy, polyploidy and pulverization. Tazima (1974), using the specific locus mutants *pe* and *re* for egg color, also found that postmeiotic germ cells of Bombyx are highly sensitive to EMS.

Larvae and pupae of Bombyx females were injected with EMS by Murakami (1975). Using the specific locus mutants *pe* and *re*, Murakami was able to show a large mutagenic effect in late pupal prophase I oocytes but not in larval growth stage oocytes (stages prior to prophase I).

Narang and Narang (1976) exposed male mosquito pupae of *Aedes polynesiense* to EMS and found inversions, translocations and deletions in brain squashes of both the treated male parent and the F_1 progeny. Their brood pattern analysis of the effect of EMS on the developing germ-cell stages indicated that dominant lethals were induced primarily in sperm and spermatids. They found no evidence in the F_1 generation for the induction of enzyme mutants detectable by electrophoresis or isoelectric focusing techniques.

Mammalian cell culture

Chinese hamster cells

Chu and Malling (1968) studied the effect of EMS on a line of V79 Chinese hamster cells. They found that EMS induced both forward and reverse mutations for 8-azaguanine resistance and reverse mutations for glutamine auxotrophy. With 8-azaguanine as the selective agent, there was no increase in the observed mutation frequency when this chemical was added to the cell cultures immediately after mutagen treatment. However, a maximum number of mutations were recorded when the 8-azaguanine was added 42 h after the removal of the EMS — a time interval permitting approximately 3 cell divisions to occur. The authors suggested that if the mutations were due to base-pair transitions, it would take at least two rounds of DNA synthesis for the mutant phenotype to appear in the population. Alternatively, the delayed expression of the mutant phenotype may have resulted from the gradual dilution of wild-type messenger-RNA or its products over several cell divisions.

It was also observed by Chu and Malling (1968) that, after EMS treatment, the observed mutation frequency to 8-azaguanine resistance was dependent on the concentration of 8-azaguanine used for selection. As the concentration increased, the observed mutation frequency decreased. The

authors suggested that each forward-mutational event represented a different isoallele with a different level of enzymatic activity controlled by the *azg* locus. Thus, at low concentrations of the selective agent, 'leaky' mutants could survive, while at higher concentrations only the non-leaky mutants would be viable.

Chasin (1973), Hsie et al. (1975), Myhr and DiPaolo (1976), and O'Neill et al. (1977) have all stressed the necessity of subculturing mutagenized cells for various periods of expression in order to obtain maximal mutation frequencies. In addition, high selective stringency appears to be important if reproducible mutation frequencies are to be obtained. Hsie et al. (1975, 1977) showed that for high selective stringency of hypoxanthine–guanine phosphoribosyl transferase (HGPRT) mutants, the selective medium should be free of hypoxanthine, and the use of 6-thioguanine is preferable to 8-azaguanine as the selective agent. Under these conditions, Shaw and Hsie (1978) demonstrated that mutation induction at the HGPRT locus was a linear function of EMS exposure (EMS concentration × treatment time) regardless of treatment time for up to 12 h. In contrast to this result, Sugiura et al. (1978) found that Chinese hamster Don cells (another strain of lung cells) showed more cytotoxic effects from EMS when the exposures where at high concentrations for a short time than when lower concentrations for a longer time were used.

Roberts et al. (1974) noted that post-treatment with caffeine potentiated the induction of 8-azaguanine-resistant mutants in V79 cells exposed to alkylating agents. Van Zeeland (1978), however, found that while caffeine potentiated killing of V79 cells exposed to EMS, it had no effect on the induction of mutations at the HGPRT locus. It was suggested by Van Zeeland (1978) that the conflicting data resulted from cell density effects during the expression time. Cell density at the beginning of selection of mutants depends on the increase in cell number during the expression time. This increase is strongly influenced by caffeine. Variation in cell density at the beginning of selection could cause differing extents of metabolic co-operation between mutant and wild-type cells and thus influence the observed mutation frequency. Since Van Zeeland (1978) respread the V79 cells after treatment with the EMS and caffeine, cell density effects should have been minimized.

After treatment with EMS or other chemicals, Bonatti et al. (1980) found higher frequencies of HGPRT-deficient mutants in V79 Chinese hamster cells grown in media containing 8-azaguanine than in media containing 6-thioguanine. Based on kinetics of expression as a function of time on the two selective media and from the results of serial selection experiments, Bonatti et al. (1980) concluded that HGPRT molecules are gradually diluted in the progeny cells of newly induced mutants, for lack of new enzyme synthesis. Resistance should be obtained only when the enzyme concentration is so low that it no longer binds to the toxic substrate. The critical enzyme concentration would be different for each selective agent since it is known that 6-thioguanine is a better substrate than 8-azaguanine (Van Diggelen et al., 1979). As a consequence, the lower number of mutants growing on 6-thioguanine could be due to the higher selective efficiency of that analog.

EMS has also been shown to be a good inducer of ouabain-resistant mutants in Chinese hamster cells (Baker et al., 1974; Arlett et al., 1975; Thacker et al., 1978). Ouabain is a specific inhibitor of the plasma membrane enzyme (Na^+/K^+)-dependent adenosine triphosphatase (Na/K-ATPase) (Dunham and Hoffman, 1970). It produces a concentration-dependent inhibition of growth in a variety of cultured cells that results from ionic imbalance in the cells (Buckland and Smith, 1976). The enzyme probably exists as a tetramer, with different parts of the molecule controlling different functions (Lea and Winter, 1977), and in the mutant cells, some of the enzyme molecules are altered so that they have lost sensitivity to ouabain but are still able to control ionic balance. It is interesting to note that mutagens such as γ-rays and ICR191 (a frameshift mutagen) give no detectable ouabain-resistant mutants, and it has been argued that these mutagens produce mostly genetic lesions leading to gross alteration in the Na/K-ATPase molecules, making potential mutants inviable because loss of ouabain sensitivity is accompanied by loss of ability to control ionic balance (Arlett et al., 1975; Lever and Seegmiller, 1976).

While EMS is able to induce ouabain resistance in mammalian cell cultures, at equal survival levels

it may produce as much as a 10-fold lower mutation frequency for ouabain resistance (Baker et al., 1974; Cole and Arlett, 1976) than for thioguanine resistance (HGPRT mutants) (Knaap and Simons, 1975; Cole and Arlett, 1976; Van Zeeland and Simons, 1976). Nonetheless, EMS is clearly able to induce ouabain resistance in mammalian cell cultures. Thacker et al. (1978) found an approximately linear relationship between EMS exposure and induced ouabain resistance in V79 cells at low EMS concentrations.

Using Chinese hamster ovary (CHO) cells, Hsie et al. (1975) showed that the frequency of EMS-induced mutations to 6-thioguanine resistance was approximately a linear function of the EMS exposure. Biochemical analyses of the 6-thioguanine-resistant variants showed that they possessed a highly reduced or undetectable level of HGPRT activity, suggesting that the EMS-induced mutations to 6-thioguanine resistance primarily affect the HGPRT locus. Couch et al. (1978) compared the effects of EMS, MMS and isopropyl methanesulfonate (iPMS) on CHO cells and found that cytotoxicity decreased with the size of the alkyl groups. All three chemicals produced a linear dose–response curve for mutations at the HGPRT locus. However, at equal levels of survival (10%) the relative mutagenic potencies of the three chemicals were: EMS > MMS > iPMS.

Abbondandolo et al. (1982) studied the induction of single-strand breaks in the DNA of Chinese hamster ovary cells after exposure to several ethylating agents, including EMS. Using alkaline sucrose gradients, they were able to measure a linear increase in single-strand breaks when the cells were exposed to EMS concentrations from 2.5 to 40 mM for 1 h. They were able to induce the same frequency of single-strand breaks with ENU concentrations 8–10 times less than with EMS. This may reflect the relatively greater amount of phosphate ethylation produced by ENU compared to EMS. The phosphotriesters formed hydrolyze in alkali (Shooter, 1976; Shooter and Merrifield, 1978) and would thereby create single-strand breaks. EMS-induced strand breaks might also have resulted from alkaline cleavage of apurinic sites arising from the hydrolysis of ethylated purines, especially *N*-7-ethylguanine.

Mouse and rat cells

Clive et al. (1972) developed a mutational assay system utilizing the thymidine kinase (TK) locus in L5178Y mouse lymphoma cells. The TK locus is diploid, and the $TK^{+/+}$ and $TK^{+/-}$ phenotypes are sensitive to 5-bromodeoxyuridine (BUdR) while $TK^{-/-}$ is resistant to BUdR. Like HGPRT, TK provides a salvage pathway that can circumvent a folate-dependent step, in this case, thymidylate synthetase. After exposing $TK^{+/-}$ cells to 10^{-2} M EMS for 2 h, Clive et al. (1972) found a 17000-fold increase over the spontaneous rate of mutations to $TK^{-/-}$. By way of comparison, 600 R of X-rays stimulated the mutation rate to $TK^{-/-}$ 2500-fold over the spontaneous rate.

Mouse L5178Y cells were used by Fischer et al. (1974) in a host-mediated assay mutagenesis system in which they looked for EMS-induced mutants that were resistant to methotrexate, cytosine arabinoside or high concentrations of thymidine. Dose–response experiments showed that mutation frequencies of all three genetic markers increased as the EMS exposure went up, both in the host-mediated assay as well as in vitro.

Tsutsui et al. (1977) showed that mutations to 8-azaguanine resistance were induced by EMS in FM38 cells from a C3H mouse mammary carcinoma, and the mutation frequency increased with higher exposures to EMS. There was a parallel increase in the frequency of chromosome aberrations in the cells with increasing EMS exposure. They were also able to demonstrate the induction of DNA single-strand breaks in the cells after EMS exposures from 0.6 to 6 mM.

Using the Jensen rat sarcoma cell line, Morrow et al. (1976) found that EMS was able to bring about an exponential increase in mutations from asparagine dependence to asparagine independence. While EMS was extremely effective as a mutagen, MNNG gave only a modest increase in the mutation frequency. These workers suggested that EMS was exerting its influence on the structural gene for asparagine synthetase by reverting a missense mutation. The involvement of a structural gene was implied from the fact that asparagine requirement is recessive in somatic cell hybrids (Morrow et al., 1973) and it results from the loss of the enzyme asparagine synthetase (Colofiore et al., 1973).

Human cells

Human lymphoblast or lymphocytoid cells have been popular in mutagenesis work because of their ready availability and indefinite life-span (Moore and McLimans, 1968). They also grow rapidly in suspension and retain a diploid or near-diploid karyotype in most of the cells of the culture (McCarthy et al., 1965; Moore et al., 1966; Zajac and Kohn, 1970; Steel, 1971). Sato et al. (1972) used a lymphoblast cell line derived from a patient with infectious mononucleosis and exposed the cells to EMS. The EMS increased the frequency of mutants resistant to 6-thioguanine more than 100-fold, to about 2×10^{-4}. Almost all of the mutants contained less than 1% normal HGPRT activity and the mutations did not appear to result from X chromosome loss. Interestingly, EMS could not produce 6-thioguanine-resistant mutants in another lymphoblast line that was used. This cell line was also considerably less sensitive to killing by EMS. The authors suggested that in the EMS-sensitive cell line there may have been an instability in the HGPRT locus, the X chromosome, or all the chromosomes as in heteroploid lines of mouse and hamster cells.

Craig-Holmes and Shaw (1977) studied the induction of SCEs in human lymphocytes exposed to EMS. A 100 μM solution of EMS produced only a slight, though statistically significant, increase in SCE frequency, while MMS induced a 3–6-fold increase at the same molar concentration. In addition, the EMS had no obvious effect on cell kinetics, while the MMS did have an effect.

Lymphoid cells derived from a case of chronic lymphocytic leukemia were found by Shiraishi and Sandberg (1979) to be highly sensitive to killing by EMS. The EMS treatment did not produce an increase in the SCE frequency but did increase the incidence of chromosome aberrations. In addition, DNA repair, as measured by unscheduled DNA synthesis (UDS) was no different in the leukemic cells and normal cells in spite of the hypersensitivity of the leukemic cells to killing by EMS.

In a comparative study between EMS and its methyl, propyl and butyl homologs, Hoppe et al. (1978) found that human diploid lymphoblasts were most sensitive to killing by MMS, followed by EMS, PMS and BMS, in that order. Thus, on an equimolar basis, there was decreasing toxicity with increasing chain length of the alkyl group. While MMS was too toxic for accurate measurement of mutations at the HGPRT locus, EMS produced a higher mutation frequency than did PMS, which, in turn, produced a higher mutation frequency than did BMS. These results most likely reflect a decreasing level of alkylation within the cells as the chain length of the alkyl methanesulfonate increases.

Human lymphoid cells have also been used to study variants of HLA, the major human histocompatibility complex, induced by exposure to EMS (Pious and Soderland, 1977; Pious et al., 1977). EMS induced dose-related increases in variant frequency of greater than 2 orders of magnitude at the highest concentrations tested. The genetic lesions induced by EMS did not extend as far as the distance between the HLA-A and -B loci (0.8 map units) in any of the clones tested and, therefore, probably represented single gene mutations.

Human diploid cells derived from a normal embryonic lung were treated with EMS by Kuroda (1974) and then scored for mutations to 8-azaguanine resistance. After treatment with 10 mM EMS for 2 h, Kuroda was able to induce resistant mutants but, as observed with other mammalian cell cultures (Chu and Malling, 1968; Chasin, 1973; Hsie et al., 1975; Myhr and DiPaolo, 1976; O'Neill et al., 1977), the mutation frequency was dependent on expression time, size of the cell inoculum, and concentration of the selective agent, 8-azaguanine.

Nelson and Harris (1978) studed EMS mutagenesis in human diploid fibroblasts using electrophoretic techniques to look for enzyme variants. They used high exposures of EMS to obtain as many mutations as possible rather than being concerned about determining the rates of mutation. From the number of clones and loci scored, it was calculated that there were 7043 chances of seeing an electrophoretic variant and 581 chances of seeing a loss of activity. A total of two mutants were identified, and the authors concluded that the yield of mutations obtained in their system was low. However, since only about 0.05% of the EMS-treated fibroblasts were able to divide and grow as clones, one has to question if the chemical dose of EMS received by the surviving fibroblasts

was the same as that received by the cells that were inactivated or killed.

By treating human diploid fibroblasts from embryonic lung with EMS, Buchwald and Ingles (1976) were able to measure induced mutations to resistance to α-amanitin. The toxic effect of this drug results from inhibition of RNA polymerase II, and the EMS-induced mutants showed RNA polymerase II activity, which was more resistant to inhibition by α-amanitin than normal. The mutants could be picked up in the diploid cells since the RNA polymerase II is synthesized codominantly. The increased resistance of the mutant RNA polymerase II to α-amatinin implies that EMS caused a DNA change that was translated into an altered amino acid sequence in the polymerase enzyme.

Genetic effects in mammals

Dominant lethals, translocations and gene mutations in germ cells

In mammals, EMS induces high frequencies of dominant-lethal mutations and translocations in postmeiotic male germ cells (Partington and Jackson, 1963; Cattanach et al., 1968; Ehling et al., 1968; Generoso and Russell, 1969), dominant lethals in female mice (Generoso, 1969; Generoso and Russell, 1969; Generoso et al., 1971) and gene mutations in postmeiotic male germ cells (Cattanach et al., 1968; Russell, 1971).

In the mouse, the frequency of EMS-induced dominant-lethal mutations reaches a peak in early spermatozoa to late spermatid stages sampled 7–10 days post-treatment. The dominant-lethal effect diminishes in earlier germ cell stages so that with a 200-mg/kg treatment with EMS, no dominant lethals above control levels are detected beyond about 16 days (Cattanach et al., 1968; Ehling et al., 1968; Generoso and Russell, 1969; Generoso et al., 1974).

Using tritium-labeled EMS, Sega and Owens (1978) were able to show that the number of ethylations per sperm head taken from the vasa deferentia at various times after injection of the labeled EMS closely paralleled the dominant-lethal frequency curve obtained with the same exposure of unlabeled EMS. Ethylation of sperm DNA was greatest at 4 h after treatment and gradually decreased during the next 2 weeks. The ethylation of sperm DNA did not increase in the germ-cell stages most sensitive to EMS and was not correlated with the dominant-lethal frequency curve for EMS. However, ethylation of sperm protamine did increase in the germ-cell stages most sensitive to EMS and showed an excellent correlation with the incidence of dominant lethals produced by EMS in the germ cells.

It was suggested by Sega and Owens (1978) that ethylation of cysteine sulfhydryl groups contained in mouse sperm protamine could block normal disulfide-bond formation, preventing proper chromatin condensation in the sperm nucleus. Stresses in the chromatin structure, possibly in the vicinity of an apurinic or apyrimidinic site caused by hydrolysis of an ethylated base, could eventually lead to chromosome breakage with resultant dominant lethality.

EMS has also been shown to induce unscheduled DNA synthesis (UDS) in meiotic and postmeiotic germ cells of male mice up to about mid-spermatid stages (Sega, 1974; Sega et al., 1976) but not in more advanced stages, even though the DNA in these later stages has been ethylated (Sega and Owens, 1978). It was noted that the germ-cell stages undergoing a UDS response to EMS were stages in which no detectable dominant-lethal mutations were induced (Sega, 1974). This led to an initial hypothesis that UDS might be important in removing the lesions responsible for dominant-lethal mutations from the germ cells. However, other mutagens, including propyl methanesulfonate (PMS), isopropyl methanesulfonate (iPMS), cyclophosphamide and X-rays were then found (Sega et al., 1976, 1978; Sotomayor et al., 1978) that produced UDS in germ-cell stages that also showed dominant-lethal mutations. The relationship between the UDS response induced in mammalian germ cells and the repair of genetic damage still remains uncertain.

While EMS induces dominant lethals, translocations and specific-locus mutations in postmeiotic germ-cell stages of male mice, none of these genetic alterations are observed in spermatogonial germ-cell stages (Ehling, 1977; Cattanach and Williams, 1971; Ehling, 1981). The absence of measurable genetic damage induced by EMS in spermatogonia may be the result of active repair of the damage or may result from selection against damaged germ

cells as they pass through spermatogonial, meiotic and postmeiotic stages.

Specific-locus tests using EMS exposures up to 400 mg/kg gave 6 mutants (scored at 7 loci) in 10491 progeny (W.L. Russell, 1971). The progeny were derived from postmeiotic germ-cell stages, mostly obtained from the first 2 weeks of mating after EMS treatment (W.L. Russell, personal communication, cited by Sega and Owens, 1978). At least 4 of the mutants were obtained with a 200-mg/kg EMS exposure. Correcting for the background mutations, the average induced mutation frequency per locus was 7.0×10^{-5}.

The induction of dominant lethals by EMS in germ cells of male mice appears to be independent of the mouse strain used (Generoso and Russell, 1969; Favor and Crenshaw, 1978). However, when female mice were treated with EMS, Generoso and Russell (1969) found that random bred T-stock females were highly sensitive to dominant-lethal induction in late oocyte stages the last few days prior to ovulation but (SEC $\times$ C57B1)F_1 and (101 $\times$ C3H)F_1 females were much less sensitive.

Soares (1976) studied the effect of the route of administration of EMS in producing dominant lethals in germ cells of male mice. No route of administration effects were found between oral and intraperitoneal (i.p.) injections regardless of the strain used. However, testicular injections of EMS did not result in induction of dominant lethals. Since the dosage in this case was based on testicular weight, only small amounts of EMS were injected per animal. Any leakage of EMS from the testes or failure to inject the EMS into the testes accurately could have produced this negative result.

In a comparative study between MMS and EMS, Arnold et al. (1976) found that MMS was effective in inducing dominant lethals in germ cells of male mice at a dose of 50 mg/kg while an EMS dose of 200 mg/kg was required to be effective. This result was in keeping with the greater chemical reactivity of MMS compared to EMS (Osterman-Golkar et al., 1970).

Ray et al. (1974) compared the exposures of EMS in mice needed to detect dominant lethals, chromosome aberrations in bone marrow metaphase cells and bacterial mutations using the host-mediated assay. Dominant-lethal effects were not evident until a dose of 150 mg/kg was used. Cytogenetic studies indicated significant breakage of somatic cell chromosomes occurred using a 115-mg/kg EMS exposure. The host-mediated assay was the most sensitive of the three procedures with a statistically significant response detectable at 35 mg/kg.

By prefeeding mice with antioxidants, such as butylated hydroxytoluene (BHT), Cumming and Walton (1973) were able to reduce EMS toxicity in male mice and were also able to reduce the frequency of dominant lethality induced in the germ cells. Since BHT is known to be a strong enzyme inducer (Creaven et al., 1966; Gilbert and Golberg, 1967), Cumming and Walton suggested that the protective effect of BHT was probably related to enzyme induction in the liver microsomes, which permitted more rapid hydrolysis of the injected EMS.

The relative sensitivities of various postcopulation precleavage and pronuclear stages to EMS were studied in the mouse by Suter and Generoso (1976). They found that EMS induced its most pronounced dominant-lethal effects when the oocytes were in the course of their second meiotic division and in early pronuclear stages. In comparison, iPMS was most effective when pronuclear formation was already completed and the majority of the zygotes were presumably undergoing DNA synthesis. It was suggested by Suter and Generoso (1976) that the difference in sensitivity patterns to EMS and iPMS could have resulted from differences in length of exposure times to the two chemicals or from differences in the mode of action of each chemical, with each having its greatest effect in a different stage.

In a study by Generoso et al. (1979) a single strain of males (101 $\times$ C3H) was treated with EMS and then mated to 4 different strains of females. The dominant-lethal frequency found using (C3H $\times$ 101) females was about 15–20% lower than that obtained with the three other strains of females. Other chemicals tested also showed differences in the induced frequency of dominant lethals in the various female strains. From these data, the authors concluded that the genotype of the egg plays a significant role in the processing of premutational lesions that are carried in the chromosomes of mutagen-treated male germ cells of mammals.

Sperm abnormalities

Changes in sperm morphology after exposing mice to EMS have been studied by Wyrobek and Bruce (1975), Moutschen and Colizzi (1975), and Bruce and Heddle (1979). In the work of Wyrobek and Bruce (1975), measurements of sperm abnormalities were made 1, 4 and 10 weeks following exposure to various doses of EMS. With an EMS exposure of 200 mg/kg, there was a significant increase in the frequency of abnormally shaped sperm at all three time points studied. Whether or not the changes produced in sperm morphology by EMS are the result of genetic alteration at specific gene loci remains uncertain. However, the fact that abnormally shaped sperm are being produced as long as 10 weeks after the exposure certainly suggests a genetic component to the effect. Bruce and Heddle (1979) also found an increase in sperm-head abnormalities 35 days after exposure to EMS. 31 days after a 20-mg/kg EMS exposure, Moutschen and Colizzi (1975) were not able to measure a significant increase in the frequency of acrosomeless mouse sperm, although there was a clear-cut increase in acrosomeless sperm after a 20-mg/kg MMS exposure. In view of the greater reactivity of MMS relative to EMS, the results most likely reflect the fact that the EMS exposure used was below the sensitivity limits of the test.

Spot test

The mouse spot test consists of treating embryos that are heterozygous at a number of specific coat-color loci, allowing animals to be born and to grow a coat, then examining this coat for mosaic patches, indicative of clones of mutant cells (L.B. Russell and Major, 1957). Using this test, Fahrig (1975) treated pregnant mice 10.5 days after conception with 50 mg/kg EMS and found the frequency of expression of one or more of the recessive coat-color genes to be about 8%. However, L.B. Russell (1977, 1978) found EMS exposures of 50 or 100 mg/kg to be only weakly mutagenic in the spot test. L.B. Russell (1977) suggested that most of the spots found by Fahrig (1975) were likely the result of killing of melanocyte precursor cells and not the result of mutational events.

Micronucleus test

In the bone marrow of mammals, micronuclei are chromatid or chromosome fragments that are left behind after expulsion of the main nucleus during maturation of erythroblasts to erythrocytes. After injecting rats with doses of EMS ranging from 100 to 250 mg/kg, Trzos et al. (1978) were able to demonstrate an increase in the number of micronucleated erythrocytes. Bruce and Heddle (1979) were able to show a similar increase in micronuclei in mouse bone marrow after EMS treatment. Thus, in addition to causing chromosome breakage in germ cells, EMS is also able to break chromosomes in mammalian blood cells.

Other effects of EMS in mammals

EMS given to pregnant rats (Hemsworth, 1968) at a dose of 200 mg/kg resulted in a high frequency ($\sim$ 62%) of limb defects in the embryos. There were also significant increases in head defects and cleft palate. In 15-day-old offspring exposed to the EMS in utero the first generation of spermatocytes was reduced to 70% of control values, while the remaining germ cells were present in normal numbers.

In rats, EMS induces tumors in kidney and brain (Swann and Magee, 1969; Montesano et al., 1974; Craddock and Frei, 1976), abdominal wall and lung (Hrushesky et al., 1972), heart (Haas et al., 1974), and mammary gland (Williams et al., 1974). EMS has also been found to induce pulmonary adenomas in the mouse (Frei, 1971). It is interesting to note that EMS is not effective in producing liver tumors in the rat, even after partial hepatectomy to induce cell division (Craddock and Frei, 1976). Since the liver appears to have high levels of DNA repair enzymes (Goth and Rajewsky, 1974; Kleihues and Margison, 1974; Nicoll et al., 1975), Craddock and Frei suggested that it may be protected from developing tumors after EMS treatment by rapid excision of lesions such as O^6-ethylguanine before mutation fixation by miscoding can take place at replication.

While EMS can be considered both a dangerous mutagenic and carcinogenic agent, it has clearly been an important chemical tool during the past 25 years for furthering our understanding of mutational mechanisms. It has served to generate many new, and often times valuable, mutants in a wide

variety of genetic test systems. The fact that the Environmental Mutagen Information Center now has over 3400 literature citations on EMS attests to the impact this chemical has had on the field of genetics and allied sciences.

Acknowledgements

I would like to thank the staff at the Environmental Mutagen Information Center, Oak Ridge, Tennessee, for their help with computerized literature searches. Special thanks go to Liz Von Halle and Eleanor Rogers for their careful editing of the manuscript. Dr. Gene Oakberg's review of the manuscript is also very much appreciated.

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