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Mutagenic Effects of some Anticancer Antibiotics

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Summary. *Considering the strong correlation observed between carcinogenic and mutagenic properties of chemicals, a study of the genetic effects of antineoplastic agents is warranted in as much as these agents may 'cure' certain types of cancers but may initiate some other types; in addition, an understanding of the effects of these antibiotics on possible transmissible alterations in the genetic material would be beneficial.*

The genetic effects of some antibiotic anticancer agents, viz., daunomycin (daunorubicin), adriamycin, bleomycin, actinomycin D, and mitomycin C are reviewed. Even though these are structurally unrelated (except for adriamycin and daunomycin), all these chemicals interfere with DNA synthesis through intercalation (daunomycin, adriamycin, and actinomycin), removal of basis from DNA (bleomycin), or co-valent bonding with DNA (mitomycin C) and strand breakage. In some cases a parallelism is found between these chemicals and radiation (e.g., mitomycin C vs UV and bleomycin vs X-rays). All these chemicals have a reversible effect on the cell cycle only when applied at low concentrations. The G_0 stage is the most resistant and the S phase, the most sensitive to these chemicals, bleomycin having a general non-phase-specific effect. Actinomycin binds to chromatin differentially along the length of chromosome, and about 90% of this binding is with the DNA. Andriamycin, daunomycin and bleomycin also bind to chromatin, but not enough is known about differential binding. Phenomena such as enzymatic reduction, e.g., in the case of anthracyclines, seem to confer tissue specificity.

The ability of actinomycin D to induce point mutations has been challenged, and most mutation-like effects appear to be epigenetic. The anthracyclines do not appear to be mutagenic either. Mitomycin C is mutagenic, however, perhaps because of its alkylating properties, as shown by studies with several species of micro-organisms, Drosophila, Habrobracon, mice, and human HLA system. Bleomycin has recently been shown to be a locus-specific mutagen in yeast.

The study of chromosomes from lymphocytes and bone marrow of individuals receiving chemotherapy, and also in vitro studies, have revealed aberrations, an effect common to all other chemicals. However, the major effect is found when the treatment affects the S phase, except with bleomycin, which also happens to be the only chemical with an effect on prophase chromosomes. Mitomycin and bleomycin also appear to effect the G_0 chromatin in mammals. The major effect of mitomycin C is the production of quadriradials by way of involving homologous chromosomes at corresponding, and preferentially heterochromatic, areas. No other chemical with such great specificity, has been found. Aberrations produced by mitomycin and actinomycin are exclusively of chromatid type, whereas others produce both chromatid- and chromosome-type aberrations — both fragments and exchanges. The aberrations are localized in a non-random fashion along the length of the genome except with bleomycin, which does not show 'hot spots' as convincingly as other chemicals do. Mitomycin C, daunomycin, and adriamycin are very potent inducers of sister chromatid exchanges (SCEs), whereas bleomycin is a poor inducer of this phenomenon. There appears to be a cell-type specificity for induction of both chromosome aberrations and sister chromatid exchanges, though it is not the same at the two end points. The study of somatic crossing-over and meiotic effects has been confined primarily to plants, except for the study of meiotic phenomena in mice and Hebrobracon and Drosophila. Again mitomycin C turns out to be the most effective chemical in these regards.

From these and other data on synergistic effects observed in a variety of organisms, there is an urgent need to draw conclusions on the effects of these chemicals on human genetic material in relation to transmissible changes, origin of neoplasia, and the control of cell growth as affected by genetic alterations brought about by these chemicals.

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Introduction

A very large number of chemicals extracted primarily from soil micro-organisms exhibit antibiotic properties. These antibiotics find applications in medicine, ranging from control of micro-organisms to promise as antineoplastic agents, and they belong to several different groups of organic molecules (Cochran and Hahn, 1975). Because man is exposed to these chemicals, knowledge of their mutagenic effects becomes important. In this article I have reviewed the effects of only the following preparations, which appear to be of some practical significance: the anthracyclines, viz., daunomycin (DNM) and adriamycin (ADM), bleomycin (BLM), actinomycin D (ACM-D), and mitomycin C (MMC). In contrast to many other antibiotics, which have their major effect on protein synthesis and are capable of differentiating between, say, bacterial and eukaryotic systems [e.g., microlide antibiotics such as carbomycin, erythromycin, leucomycin, kujimycin, spiramycin, etc. (Vasquez, 1975)], the antitumor agents appear to have a definite direct effect on nucleic acids. Perhaps it is this one property of these chemicals that makes them effective against a variety of cancers in man (See Carter, 1976; and Table 1).

The target cells and modes of action of these chemicals differ widely. BLM, which is a mixture of related compounds, has a particular affinity for squamous-cell carcinoma and epithelial tissues. Perhaps tissue selectivity is partly due to the selective concentration of the drug in the tissue and partly due to poorer ability to detoxify the chemical enzymatically (Connors, 1975). In contrast, anthracyclines are enzymatically reduced to duanomycinol (Bachur and Huffman, 1971; Huffman and Bachur, 1972), which is probably a more selective antitumor agent. In this case, antitumor effectiveness has been correlated with the levels of reductase. The anthracyclines and ACM-D appear to act by some form of intercalation, thus interfering with replication and transcription. Cardiac toxicology, however, is the major problem in the use of anthracyclines. It has been suggested that the main mechanism of action of MMC on the cancer cells is alkylation. It appears that similar or identical actions of these chemicals, which are the basis for chemotherapy, are also responsible for the genetic effects they produce. Such effects of some of these

Table 1. Major types of cancers that respond to antibiotics discussed in the present review[a]

Antibiotic	Postulated mechanism of action and dose	Cancer type	Response rate
Daunomycin	Inhibits DNA-dependent RNA synthesis Dose 30–60 mg/m²/week		
Adriamycin	Intercalates between base pairs of DNA, inhibits DNA-dependent RNA synthesis Dose 60–90 mg/m², with total dose of 500 mg/m²	Breast Lung Small-cell type Squamous-cell type Stomach Testicular Ovarian Cervical Head and neck	35% 25% 19% 26% 20% 38% 32% 19%
Bleomycin	Strand scission of DNA; Dose 10–15 mg/m²/week to a total of 300–400 mg	Lung Squamous-cell type Testicular Cervical Head and neck	 13% 52% 10% 15%
Actinomycin	Forms stable complex with DNA; inhibits RNA synthesis Dose 0.5–1.5 mg/m²/week for 3–5 weeks	Testicular	52%
Mitomycin	Probably alkylating agent Dose 10–20 mg/m² every 3–8 weeks	Breast Colon Stomach	37% 16% 30%

[a] Data from Carter (1976) and WHO (1977)

chemicals have been extensively reviewed elsewhere (Vig, 1977; Vig and Lewis, 1979).

Chemistry and Physical Interaction with Macromolecules

The compounds being considered in this review are uniform in neither structure nor physicochemical properties. Historically, ACMs were the first of these antibiotics to be discovered, in 1940. These were isolated from the fungus *Actinomycetes* by Waksman and Woodruff (1940). These are also the first of the series of peptide-containing pigmented antibiotics (Fig. 1). ACM-D is the most extensively studied of these. Considering the fact that this drug is highly toxic, has a low therapeutic index, and was not subjected to any serious biochemical study until the late 1950s, the amount of attention given to it recently is impressive. This agent is a competitive inhibitor of pantothenate, and the first phenomenon to be inhibited is protein synthesis (Foley, 1956). However, the ability of the drug to inhibit nucleic acid synthesis was not known until 1960, when Kirk (1960) showed that RNA synthesis is inhibited by this drug much more rapidly than protein or DNA synthesis. It is now known that cellular RNA synthesis inhibition by ACM-D is about 100 times more sensitive than plasmid RNA synthesis (Ehling, 1974). The observation that DNA synthesis inhibition can be reversed by the addition of excess DNA (Goldberg et al., 1962) suggested that the initial binding site might be at the level of DNA. Support for this model has been provided by the finding that chromophore binds to guanine residue (Kirk, 1960), and by Lerman's idea of intercalation of the acridine molecule to bind to DNA (Lerman, 1961). The stereochemistry of ACM-DNA interaction has been dealt with excellently by Sobell (1973). This intercalation has been well documented by increased internal viscosity, spectral changes, kinetics of association and disassociation of ACM from DNA, and changes in the sedimentation behavior of DNA.

The ACM-induced changes in physical properties of template DNA may cause inhibition of RNA polymerase and DNA polymerase (Reich, 1964) but cannot explain the selective inhibition of certain species of RNA (Rickenson, 1970; Tsuboi and Terasima, 1970) or stage-specific RNA-transfer sensitivity, say, in *Ornithogalum virens* (Church, 1973).

MMC (Fig. 2) was isolated from *Streptomyes caespitosis* (Kirsch, 1977) in 1956, and was shown (Shiba et al., 1959) to be a specific inhibitor of DNA synthesis. It is now generally recognized that this antibiotic acts as an alkylating agent (Schwartz et al., 1963). Iyer and Szybalsky (1963) have since shown that MMC creates cross-links of complementary strands of DNA resulting

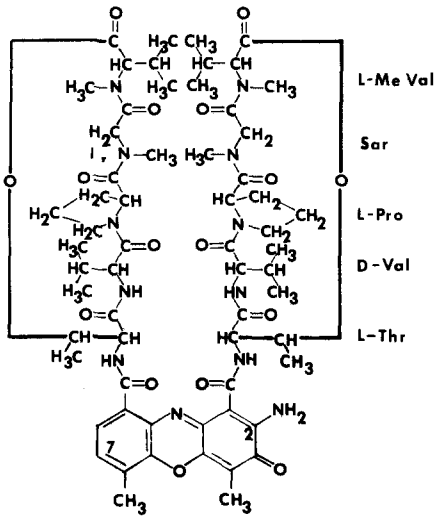

Fig. 1. General molecular structure of actinomycins

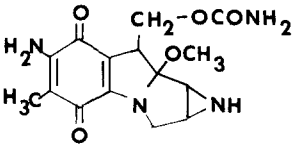

Fig. 2. General molecular structure of mitomycin

from co-valent bonding with DNA, after apparent reduction of the antibiotic to its hydroquinone derivative, however (Samuels, 1964). DNA binding, so well correlated with cell death (Szybalski and Iyer, 1964), is dependent upon the presence or absence of proteins associated with DNA. In bacterial DNA the MMC-DNA binding is close to 100% (Waring, 1968), in contrast to poor binding in DNP-rich chromatin of human tissue (Szybalski and Iyer, 1964; Szybalski, 1965). This interaction results in changes in physical properties of DNA, e.g., UV hypochromicity, transforming ability, and buoyant density. The inhibition of DNA polymerase but not of RNA polymerase by MMC has been explained by the action of the molecule on DNA but lack of binding with single-stranded RNA, as also noticed for single-stranded DNA of $\varnothing \times 174$ (Kersten et al., 1964).

DNM and ADM are the most effective antibiotics used in cancer chemotherapy. These were isolated, respectively, in 1963 and 1969 from *Streptomyces peucetius* var. *caesius* (Carter et al., 1972; DiMarco et al., 1975; Vig, 1977). The chromophores (Fig. 3) differ from each other in that the H at position 14 in DNM is replaced by an OH in ADM. This substitution makes ADM a more effective (about 80%) chemotherapeutic drug than DNM (DiMarco et al., 1969). These anthracyclines interfere with DNA template function through intercalation, thus causing usual changes in physical properties (e.g., reduction of antibiotic fluorescence, Δ

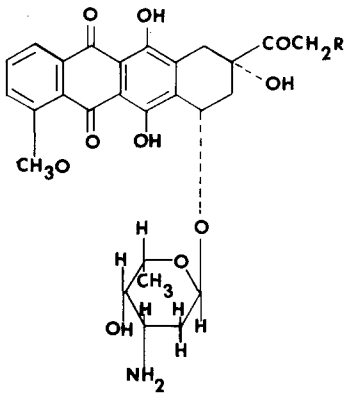

Fig. 3. General molecular structure of anthracyclines

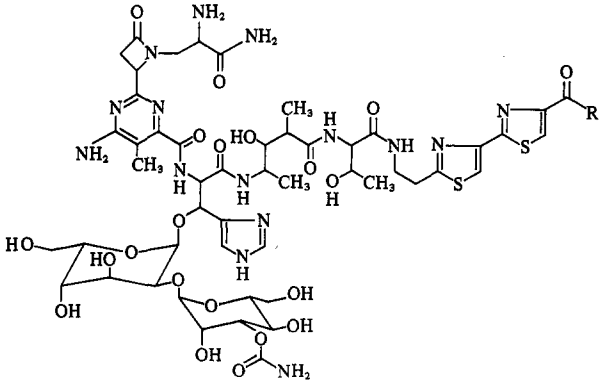

Fig. 4. General molecular structure of bleomycins

Tm, decrease in buoyant density of DNA, Δ intrinsic volocity and protection from nucleolytic enzymes). Besides electrostatic interaction involving one PO_4 of DNA and an OH group of anthracycline, the amino sugar of anthracyclines also interacts with the second DNA phosphate (Pigram et al., 1972). Not only are the binding sites for ADM and ACM-D different, as the former do not show any preference for the G : C base pairs, but also, anthracycline treatment of DNA increases the ADM-DNA binding (Krugh and Young, 1977). These molecules inhibit DNA-dependent RNA synthesis and RNA and DNA polymerases (DiMarco, 1968; Goodman et al., 1974; Zunino et al., 1975). The RNA synthesis inhibition is more severe for nuclear, than for extranuclear, RNA (Silvestrini et al., 1970). In addition, these chemicals can inhibit reverse transcriptase activity (DiMarco et al., 1975), but no intercalation is found on single-stranded DNA and RNA (Calendi et al., 1965; Cohen et al., 1969).

BLM (Fig. 4) is the most recent of the anticancer antibiotics under consideration. It was isolated in 1966 by Umezawa et al. (Umezawa, 1975) from *Streptomyces verticillus*. BLM is a mixture of several related compounds, e.g., A_1, $A_2 \ldots A_6$ and B_1, $B_2 \ldots B_5$, generally differing in the terminal amine moiety. In reaction mixtures containing mercaptoethanol and hydrogen peroxide, BLM decreases the Tm of DNA, particularly of poly d(G), poly d(C). When this antibiotic is preincubated with template DNA, the DNA-dependent RNA polymerase reaction is inhibited (Tanaka, 1970). On the other hand, BLM is known to cause stimulation of DNAase activity. In addition, BLM does not inhibit DNA-dependent DNA polymerase activity in *E. coli* and mouse liver cells (Umezawa, 1975).

Antibiotic-induced Damage to DNA

ACM-D can cause breakage of DNA (Pater, 1974) in both normal and xeroderma pigmentosum cells (Kleijer et al., 1973), and can inhibit repair replication (Cleaver, 1969; Humphrey et al., 1970) in irradiated cells. However, there are contrary reports for mouse leukemia cells (Kahn et al., 1974; Lee et al., 1972). Could it mean that the repair process as effected by ACM-D is a function of cell type? Additionally, a concentration-dependent enhancement of BLM-induced DNA fragmentation by ACM has been reported (Bearden and Haidle, 1975).

The main action of MMC is cross-linking of the complementary strands of DNA. Even though the action is by monofunctional alkylation, it has been suggested that the effect of this chemical on DNA is similar to that of UV radiation. This idea was strengthened with the finding that MMC-resistant cells exhibit cross-resistance to UV (Otsuji and Murayama, 1972), and repair-deficient cells, e.g., those homozygous for the gene for Fanconi's anemia, show enhanced sensitivity to MMC as well as to UV. This suggests that the enzymatic background necessary for the induction of cross-links by MMC and UV is similarly affected. This observation, however, is contrasted with the observation that *v* gene-mediated repair in *E. coli* B^{s-1} genome (Friedberg, 1972) shows no response to MMC-induced damage, whereas UV-induced damage is repaired. A similar lack of parallelism is found for UV-induced reversions to Trp^+ – MMC is incapable of inducing such change.

BLM inhibits incorporation of ^{3}H-TdR into DNA in intact cells and causes pH-dependent single-strand breakage of DNA (Kohn and Ewig, 1976). Such fragmentation in treated cells is rapidly repaired when cells are grown in BLM-free medium (Byfield et al., 1976; Iqbal et al., 1976). It is proposed (Jørgensen, 1972) that the BLM effect is enhanced by simultaneous X-irradiation. It is concluded (Clarkson and Humphrey, 1976; Terasima and Umezawa, 1970) that BLM-induced lesions in DNA are finally converted to single-strand breaks. Apparently the chemical is activated by dithiothrietal to an intermediate stage, with an ability to interact with DNA (Muller et al., 1972).

The damage induced to DNA by anthracyclines takes the form of both single- and double-strand breaks (Byfield et al., 1977). The concomitant effect on enzyme production appears to lead to irreparable strand breakage. Byfield's observation that damage induced to DNA by ADM is similar to that induced by X-rays, is intriguing (Byfield et al., 1977), but needs further support. However, the ADM effect is additive to that of X-rays at high levels of cell survival but synergistic at low levels of cell survival, even though ADM does not appear to inhibit the repair of lethal X-ray damage (Byfield et al., 1977).

Effect on Cell Cycle

All antibiotics considered in these studies appear to have reversible effects on the cell cycle progression of eukaryotic cells at low concentrations only. In none of the studies has a precise stage been identified as the most sensitive, even though the G_0 stage is recognized as the most resistant.

The generalities that emerge from studies with ACM-D indicate that the G_1 and G_2 phases of mammalian cell cycle are uniformly more sensitive than the S phase; treatment of cells early in G_1 may delay their entry into the S phase (Defoor and Stubblefield 1900), perhaps because some proteins essential for DNA synthesis are not synthesized (Epstein et al., 1972). Similarly Tobey (1975) has concluded the presence of several 'transmission points' in G_2 that may be critical for a cell to proceed to ensuing mitosis. Even though there are reports (Bal and Ste-Marie, 1969) of sensitivity of early S cells, late S cells are usually not affected (Epstein et al., 1972). There is also evidence that inhibition of nucleolar specific RNA synthesis may block G_1 or G_2 cells (Baxter and Byyoit, 1975; Rickenson, 1970) but that DNA synthesis, once initiated, continues at uninterrupted rate (Rickenson, 1970).

For MMC, the data are more conflicting. Cells in as well as out of the S phase are affected (Mihich, 1973). In one study HeLa cells showed high sensitivity to MMC from the second half of G_1 to the first half of S, but in another (Djordjevic and Kim, 1968) this was more pronounced for non-S-phase cells than for those in the S phase. In the colony-forming cells of mouse bone marrow the antibiotic has a more pronounced lethal effect on cells in the S phase than in G_1. There is little, if any, effect on G_2.

Whereas anthracyclines can cause blockage of cells in both G_1 and G_2 (DiMarco et al., 1975; Friedberg, 1972; Wheatley, 1971), the major effect, as determined by chromosome aberration studies (Vig, 1977), and cell cycle kinetics studies (DiMacro et al., 1975), is definitely in the S phase. The inhibition of RNA synthesis in the preceding G_1 phase (Evanson, 1967) also seems to affect the cell later in S phase. Interference with DNA synthesis does not appear to be the only effect of anthracyclines, since doses lower than those that affect DNA directly have been shown to cause changes in the cell cycle progression rate (Vig, 1977).

Whereas BLM is capable of blocking cells at G_1 as well as at G_2, the effect does not appear to be uniform. Thus, a proportion of the treated G_2 population may proceed on to the next cell cycle without undergoing cytokinesis (Nagatsu et al., 1972). It is known (Barranco and Humphrey, 1971; Clarkson and Humphrey, 1976) that Chinese hamster cells in mitosis are increasingly more sensitive to BLM than those in G_2, early S, late S, or G_1. However, Terasima and Umezawa (1970) found HeLa cells to express most sensitivity during G_1 and least in G_2. The effect of BLM on mammalian cells appears to be similar to that of X-rays (Byfield et al., 1977; Jørgensen, 1972). Accumulation of BLM-treated Ehrlich ascites carcinoma cells in G_2 has also been reported (Promchainant, 1975).

Binding to Chromatin

ACM-D is the only molecule whose binding to chromatin has been studied in any detail. It is found to bind to both native and isolated DNP complex, about 90% of this binding being between ACM-D and DNA (Alam and Steel, 1973). The binding is restricted to double helical DNA (Hyman and Davidson, 1967) and does not appear to depend exclusively upon GpG sequence. The binding increases from metaphase to G_1, with constant binding during most of the S phase, but declines towards the late S phase (Pigram et al., 1972). Isolated chromatin binds much more ACM than native DNA, suggesting that increased binding depends upon the removal of proteins (Beato et al., 1970). As expected, the binding coefficient varies with the cell type (Barcellona et al., 1974; Brachet and Hulin, 1970; Thaler et al., 1969) and it has been suggested that it is a function of plasma membrane (Biedler and Riehm, 1970), ionic concentration (Bolund, 1970), and treatment of chromatin (Beato et al., 1970).

Different regions of chromosomes bind ACM-D differentially. In human chromosomes visualized at metaphase, the telomeric and centromeric regions show enhanced binding (Miles, 1970). In *Vicia* (Cionini, 1973) least binding is found on regions that express intense quinacridine fluorescence, whereas intense bands occur at ribosomal cistrons located at nucleolar organizing regions (Cionini and Avanzi, 1972). Such studies do not consistently show that heterochromatic regions bind ADM more intensely than euchromatin. Even heterochromatins in *Vicia* and *Phaseolus* show differential

binding, suggesting basic structural differences (Cionini and Avanzi, 1972; Cionini, 1973) or functions of various sets or regions of chromosome (Berlowitz et al., 1969). Differences in this regard are known from studies with mealy bug (Berlowitz et al., 1969), hamster (Simard, 1967), *Microtus* (Sieger et al., 1971) and avian (Seligy and Lurquin, 1973) chromatin. Transcriptionally active chromatin, due to temporary release from protein, appears to bind more strongly to ACM-D than inactive chromatin. Examples are available from studies with avian (Seligy and Lurquin, 1973), *Asellus* (Rocchi et al., 1974), and human lymphocytes (Ringertz et al., 1969) and calf thymus (Pospelov and Pupyshev, 1973) chromatin. The observation that stepwise deproteinization of chromatin increases ACM-DNA binding suggests that the DNA complex does not attach to ACM molecule as long as proteins are associated with DNA (Berlowitz et al., 1969; Sieger et al., 1971). This does not mean, however, that the biological activity of the molecule depends upon its binding intensity, as has been shown for ACM-D and ACM-F, which show similar binding to DNA (Cavalieri and Nemchin, 1968) but widely different activities.

Apparently no such studies have been done with MMC. Information available for anthracyclines indicates a strong affinity for chromatin, since in intact cells ADM is found almost exclusively in the nucleus (DiMarco, 1968) and specifically in DNP fraction (Calendi et al., 1965). However, Krishan et al. (1976) have recently shown that *N*-trifluoro-acetyladriamycin-14-valerate, a potent anthracycline, limits its distribution to cytoplasm. It is also known that DNM is taken up by the cell throughout its cycle of division (Silvestrini et al., 1970), maximum uptake occurring in the S phase (DiMarco et al., 1975). For BLM, the brief reports on binding (Fujimoto et al., 1976) show that the molecule can bind to nucleoprotein complex and label can be detected on metaphase chromosomes.

Genetic Effects

1. Induction of Point Mutations and Epigenetic Changes

In an attempt to correlate 'carcinogenicity with mutagenicity' and 'antineoplasticity with antimutagenicity,' Szybalski found ACM-D was ineffective as a mutagen in a streptomycin-dependent strain, SD-4, of *E. coli* (Szybalski, 1965). This anticancer agent has subsequently been shown to be nonmutagenic in *Drosophila* (Mukherjee, 1965) but capable of inducing mutations at the *AD-3* locus of *Neurospora* (Fisher et al., 1975).

Certain effects produced by ACM-D have turned out to be epigenetic. In sea urchin (Gross and Cousineau, 1964; Infante and Nemer, 1967), amphibia, and chicks (Brachet and Hulin, 1970) such effects lead to developmental anomalies, and in *Ilyanassa obsoleta* stage-specific differentiation is blocked. Margulies (1972) has utilized such an approach to point out that some genes affecting differentiation of imaginal discs are activated in the third larval instar in *Drosophila,* in similar studies to those establishing some sequences of gene action in the planarium *Dugesia gonocephala* (Coward, 1968). In polytene chromosomes of *Drosophila hydei* ACM-D inhibits puff regression induced by ecdysone (Ashburner, 1972) and prevents the recovery of heat-induced puffing changes. In ameba, ACM-D-induced 'hereditary' changes may persist for several generations (Kalinina, 1968), and such changes may induce phenotypic reversions in *Neurospara* (Fisher et al., 1975) and phenocopies in *Drosophila* (Margulies, 1972).

Mutagenic effects of anthracyclines have been studied for ADM, DNM and aclacinomycin. In Ames' studies the *Salmonella typhimurium* tests have shown weak mutagenicity of ADM and DNM (McCann et al., 1975). Aclacinomycin appears to be negative in this regard. However, in our laboratory and in that of Anwar (unpublished), whereas DNM and ADM generally proved to be potent chromosome-breaking agents, these are poor to totally inactive point mutagens in *Salmonella* and yeast. From this point alone, these chemicals are interesting in the study of genetic effects. To this should be added the cases of BLM and DNM, which are also nonmutagenic in yeast (Hannan and Nasim, 1978).

The anticancer antibiotic most extensively studied for its mutagenic properties is the alkylating agent MMC. It is perhaps the alkylation that makes it the most effective mutagen in this class of compounds. In 1958, Szybalski demonstrated its mutation-inducing ability in *E. coli* (Szybalski, 1965) for lac^+ to lac^- and for arg^+ to arg^-, and his^+ to his^- mutations. A variety of mutational events produced by MMC (Green et al., 1976; McCann et al., 1975; Tsan, 1970) can be attributed to its capacity to attack DNA randomly and its effect on one or both strands (Tsan, 1970). Some of these mutations may be attributed to small deletions, as has been postulated for *gal-chl* region of *S. typhimurium* (Alper and Ames, 1975) or *E. coli* (Ishii and Kondo, 1975).

Some strains of *E. coli*, e.g., K-12, are sensitive to MMC (Imae, 1968), while others, like B/r, are highly resistant (Ikeda and Iijima, 1965). Strains showing efficiency and accuracy of recombination repair (e.g., *Micrococcus radiodurans* (Sweet and Moseley, 1976), show extreme resistance, and exc^- and $recA^-$ mutants of *E. coli* appear to be resistant; unexcised lesions may lead to the death of affected cells (Latt, 1974) even though strains defective in DNA polymerase I are found to be highly mutable (Ishii and Kondo, 1975). The re-

combination repair-deficient mutants in *E. coli* and *Bacillus subtius* seem to fall into more than one category, since steps involved in DNA repair in these mutants are different in nature (Ishii and Kondo, 1975, 1974; Pekhav and Rudneva, 1970).

Among higher organisms, mutagenicity of MMC has been well documented in Drosophila (Mukherjee, 1965; Schewe et al., 1971; Suzuki, 1970). About 3% of all sex-linked mutants are conditional lethals showing temperature sensitivity and turn out to be a genetically more homogeneous group than similar mutants induced by radiation. In addition, MMC appears to differ from other alkylating agents, e.g., EMS, in the spectrum of mutations induced. MMC-induced temperature-sensitive mutants are also found in *Habrobracon* (Smith, 1969). In silkworm a 'storage effect' appears to be pronounced in the case of MMC-induced mutations, in that the effect is increased with reduced storage periods (Inagaki and Oster, 1969). True mutations have been observed only for spermatogonia in mice treated with MMC (Ehling, 1974). Recently Pious et al. (1977) have demonstrated a tenfold increase over control values in the induction of HLA mutations in human lymphoid cells treated with MMC.

2. *Induction of Chromosome Breakage*

The most extensively studied genetic effect of the chemicals under review is their ability to induce structural damage to chromosomes both in vitro and in vivo. All these chemicals induce chromosome breakage in mammalian cells, and the most sensitive phase of the cell cycle appears to be S. The effect may not depend entirely upon DNA synthesis or its inhibition, however, since during this phase chromosomal proteins are synthesized in much larger quantities than in the G_1 and G_2 phases, which are also affected by these chemicals, except for MMC. All chemicals are capable of expressing a delayed effect. Even low concentrations can drastically affect both the frequency of cells with aberrations and the aberration frequency per cell. A few examples are given in Table 2.

From a qualitative point of view, there are interesting differences in the end effects produced by these chemicals. ADM and DNM, for instance, produce both chromosome- and chromatid-type aberrations within one cell cycle. MMC produces quadriradials involving homologues at apparently identical positions, thus creating configurations that appear to be forerunners of so-

Table 2. Some examples of in vitro effects of anticancer antibiotics on mitotic chromosomes of some animal and plant species

	Tissue	Treatment (μg/ml)	Recovery (h)	Aberrant mitosis %	Aberrations per cell[a]	Ref.
ADM	Human lymphocytes	0.1–0.5 for 72 h	0	74–90	–	134
	Fibroblasts	0.01–0.05 for 1 h	5–12	42–80	0.6–2.38	154
	Lymphocytes	0.02 for 24 h	0	64	0.82	154
	Lymphocytes	0.05–0.15 for 4 h	24 or 48	50–82	1.06–3.83	219
	Lymphocytes	0.02 for 24 h	0	84	3.03	219
DNM	Human lymphocytes	0.2–0.6 for 24–72 h	0	41–92	–	81
	Medullary cells	0.2 for 72 h	0	82	–	81
	Lymphocytes	0.1–0.16 for 36 h	24	17–41	0.38–0.81	229
MMC	Human lymphocytes	0.01 for 24 h	0	24	0.22	182
	Human lymphocytes, FA^+	0.01 for 24 h	0	93–100	0.27–0.74	182
	Fibroblasts	5.0–50 for 18 h	0	–	–	74
	Lymphocytes	0.1–5 for 24 h	0	–	–	46
	Tradescantia paludosa	10 for 1 h	24–72	1–3.5	0.05–0.14	216
	Vicia faba	10 for 1 h	24–72	2–8	0.03–0.31	216
	Vicia faba	5 for 2 h	12–18	12–38	0.29–0.41	186
ACTD	Human lymphocytes, SKL-1, LK 63	0.2–0.4 for 2–4 h	0	–	0.08–2.5	138
	Lymphocytes	0.02 for 24 h	0	22	0.03	182
	Lymphocytes, FA	0.02 for 24 h	0	85	1.63	182
BLM	Human HeLa	1–100 for 4–24 h	24	–	1.42–11.11A	161
	CCRF.CC1	1–100 for 20 h	24	–	0.80–2.97A	161
	Mouse Fibroblasts	0.25–25 for 4–20 h	24	–	0.66–16.97A	161

[a] Does not include gaps or achromatic lesions, + FA = from patients with Fanconi's anemia
A = Per 100 chromosomes

matic recombination. Other chemicals do not do so, even though anthracyclines produce a considerable number of exchanges between homologues at noncorresponding loci.

2.1. In Vitro Effects. a) Mitomycin C: Using MMC for the first time, Merz (1961) showed that root tips of *Vicia faba,* even when treated for only 1 h in 0.001% solution, produced all types of chromatid aberrations. The action is delayed, rejoining occurs, and the aberration frequency is not affected by anoxia, changes in pH, or temperature (Cohen and Hirschhorn, 1971; Kihlman, 1960; Merz, 1961). However, whereas the effect is known to be limited to the S phase in *Vicia,* mammalian cells show effects in the G_0, G_1, or S phase (Nowell, 1964). The chromosome-breaking capacity has been attributed to the induction of cross-links by MMC and hence exclusive production of chromatid-type aberrations.

Aberrations induced by MMC are limited mainly to heterochromatic regions or secondary constrictions. A comparison between *Vicia faba* genome and *Nigella damescena* and *Tradescantia paludosa* genomes (which lack visible heterochromatic blocks) proves this point convincingly (Natarajan and Ahnstrom, 1969; Utsumi, 1971). Natarajan has shown that in mouse 70%–80% of all aberrations are localized in the heterochromatin, which makes up only 10% of the genome (Natarajan and Raposa, 1975). This observation, however, dispenses with the old idea that MMC preferentially attacks G : C-rich regions, since constitutive heterochromatin of the mouse is composed mainly of A : T base composition (Flamm et al., 1969; Yasmineh and Yunis, 1970). Natarajan and Schmidt (1971) further demonstrated that the MMC effect is due primarily to the structural rather than functional properties of heterochromatin, even though the long arm of the inactivated X chromosome in Chinese hamster is affected more frequently than the long arm of active X.

A striking effect of MMC is the induction of chromatid exchanges involving homologues at corresponding points producing quadriradials (German and LaRock, 1964). Such observations have been confirmed in human leukocytes (Clewell and Evenchik, 1973; German and LaRock, 1964; Nowell, 1964) and *Vicia faba* (Rao and Natarajan, 1967; Shah et al., 1972), and are suggestive of genetic crossing-over. Comings (1975) has proposed that the basic cause of such exchanges is repetitive sequences in the constitutive chromatin regions producing DNA hybrids. A somewhat similar opinion had previously been expressed by Brogger (Brogger and Johansen, 1972), who also provided ideas for the production of free fragments. The analysis of the *homo/het* ratio of MMC-induced chromatid exchanges in *Vicia faba* (Rieger et al., 1973) is strongly suggestive of pairing of somatic chromosomes in interphase, at least to the extent that it supports the concept of fusion tendency of heterochromatin (Natarajan and Ahnstrom, 1969) as a strong factor in producing exchanges. These observations, though, do not provide answers to the question as to why one finds a far higher frequency of interchanges in the X-chromosome of *Microtus agrestis* than X-X translocations in cells treated with MMC (Natarajan et al., 1974), especially in view of the constitutively heterochromatic nature of X chromosome in this organism (Arrighi et al., 1970). In studies by Morad et al. (1973), nonrandomly distributed exchanges induced by MMC were overrepresented in the C bands of homologues. In *Vicia,* clustering of aberrations occurs within or adjacent to G bands and breaks and exchanges do not share the hot spots to the same extent. Generally, the distribution of breakpoints differs from Poisson distribution but does tend to agree with what Bochkov calls the geometric pattern (Bochkov et al., 1972). This pattern is independent of the stage of cell cycle and the time of fixation.

b) Actinomycin D: Several organisms, e.g., human cells (Gross and Coustineau, 1964; Ostertag and Kersten, 1965; Voorhees et al., 1969), *Vicia faba* (Ward and Glover, 1969), mouse (Jain and Singh, 1967; Manna and Mitra, 1971), Indian muntjack (Pathak et al., 1970), and locust (Jain and Singh, 1967) have been tested for the chromosome-breaking ability of ACM-D. The aberrations induced are almost entirely of the chromatid type and, even though distributed somewhat randomly, show a preponderance of localization in heterochromatic regions, e.g., pericentric regions (Ostertag and Kersten, 1965) and secondary constrictions (Ward and Glover, 1969), including nucleolar organizing regions (Pathak et al., 1975). In one study (Manna and Mitra, 1971), 90% of ACM-D-induced aberrations in mouse were found to be localized in regions other than proximal heterochromatins – a finding lacking conformity with the MMC-induced aberrations. Broken ends of chromosomes appear to reunite in every study in mitotic chromosomes; however, in meiotic chromosomes of locust, this feature appeared to have been adversely affected (Jain and Singh, 1967), but not in mouse (Jagiello, 1969). A large proportion of breaks usually show rejoining – up to 40% in one instance (Ostertag and Kersten, 1965).

ADM-D is most effective in the S phase (Miles, 1970; Ostertag and Kersten, 1965). However, some minor effect can also be found in post-S i.e., G_2 cells (Manna and Mitra, 1971; Miles, 1970; Ostertag and Kersten, 1965). At a high concentration, e.g., 5 μg/ml for 2.8 h, most chromosomes in the Chinese hamster genome were decondensed or stretched, leaving small metacentric chromosomes, the heterochromatic Y, and

the X intact (Arrighi and Hsu, 1965). This has been taken as evidence of the ACM molecule interfering with the histone-DNA binding of some chromosomes. Such a conclusion does not agree with the data obtained from binding studies of ACM-D with the chromosomal region. One must consider that binding sites of the ACM-D molecule in human chromosomes do not agree with the points of breaks induced by the antibiotic in chromosomes (Miles, 1970).

c) Bleomycin: Chromosome aberrations induced by BLM were first reported by Ohama and Kadotani (1970), who observed a high incidence of chromatid gaps, breaks, fragments, and translocations in human lymphocytes. Pulverization of Chinese hamster chromosomes has also been observed (Ikewchi, 1973). In one study dealing with cultured mouse fibroblasts and human cells of neoplastic origin, increased concentrations resulted in a reduced overall frequency of aberrations (Paika and Krishan, 1973), indicating that only a resistant subpopulation progressed under the influence of high concentrations. A point of interest that emerges from some studies (Tamura et al., 1974) is the increased incidence of aneuploidy, perhaps related to the observation that DNA synthesis in BLM-treated cells can continue from 2C to 4C or 8C values without nucleokinesis (Nagatsu et al., 1971, 1972).

It has been suggested that BLM is capable of affecting G_1 cells so that both chromatid and chromosome aberrations are produced, signifying that both single- and double-strand breaks can be induced in DNA. Whereas the S phase appears to be the universally sensitive stage for induction of aberrations, the report that not only fragments (Hittleman and Rao, 1974; Paika and Krishan, 1973) but also exchanges can be produced by affecting G_2 cells (Paika and Krishan, 1973) remains to be confirmed.

Studies in human lymphocytes indicate a nonrandom effect of the drug on chromosomes. The large chromosomes appear to be most affected, with almost no effect on chromosomes of group F, G, or Y (Promchainant, 1975). Such observations on lack of effect differ somewhat from those reported for other drugs in these studies.

To the knowledge of the author, there are no reports of chromosome aberrations induced by BLM in plant materials. However, Moutschen et al. (1973) showed that the drug is capable of potentiating such effects of X-rays in *Nigella damascena*. The potentiation factor increased with either higher doses of BLM or radiation, and presoaked seeds expressed higher potentiation than dry seeds.

d) Anthracyclines: ADM and DNM cause extensive damage of chromosomes in human leukocyte cultures (Grouchy and DeNova, 1967; Massimo et al., 1970; Newsome and Littlefield, 1975; Vig et al., 1968a and b), human medullary cells (Grouchy and DeNova, 1967), Chinese hamster cells (Hittleman and Rao, 1975), rat bone marrow (Jensen and Philip, 1971), and mice (Stromskaya and Pogosianz, 1971). The first details were provided by Grouchy (Grouchy and DeNova et al., 1967) and Vig (Vig et al., 1968a and b) for DNM and by Vig (1971) and Massimo (Massimo et al., 1970) for ADM. Whereas both chemicals are capable of inducing breaks and exchanges of both chromatid and chromosome types, the interesting aspect is the production of both types of exchanges in the same cell (Vig et al., 1968). These observations have been confirmed by other laboratories (Hsu et al., 1975), and indicate that the molecule is capable of acting on both the single-stranded and the post-duplicate, double-stranded, chromosome. Triradials, generally so rarely found, are found rather commonly in anthracycline-treated cells, again indicating the possibility of chromatid and chromosome aberrations in the same cell (Vig, 1971).

Molecular alterations in the parent anthracycline molecule do not always retain the ability to induce genetic damage. The *N*-acetyl derivatives of DNM and aclacinomycin do not induce chromosome damage in lymphocytes.

Aberrations induced by anthracyclines are nonrandomly distributed along the length of the genome. Thus in human chromosomes, pairs 1, 2, 3, 4, 5, 6–12/X and 13/15 show a higher frequency of breaks than expected, whereas others show deficiency (Vig, 1971; Vig et al., 1970). These results differ from those obtained with MMC in that break points and exchange points overlap and that clustering is not specifically limited to C chromatin. However, recent results obtained by Cornforth (unpublished) with mouse cells show that the sensitivity of constitutive heterochromatin is dependent upon the part of the cell cycle in which ADM is administered.

A dividing cell is sensitive to anthracyclines in all parts of interphase. The G_0 phase, however, has been found to be insensitive in that freshly cultured human lymphocytes do not respond to treatment for about the first 14 h (Vig et al., 1969). Not only does sensitivity increase as G_1 advances, but also a large proportion of aberrations enter exchange formation (Vig et al., 1969). In some studies (Newsome and Littlefield, 1975; Vig, 1978) very few aberrations appear as exchanges, perhaps because of a lower total frequency of lesions.

Chromosome aberrations are also produced in G_2 cells in routine cultures (Sinkus, 1972) and with the technique of premature chromosome condensation (Hittleman and Rao, 1975). In addition, whereas application of FudR does not seem to alter the frequency or nature of aberrations induced by DNM, puromycin does increase the frequency of free fragments at the ex-

pense of exchanges (Vig, 1970), thus indicating that DNA synthesis per se may not be a prerequisite for expression of DNM-induced aberrations. Similarly, the chromosome-breaking effect of ADM is potentiated several-fold by treating the cells at 43° as against 37° C. Lowering the temperature reduces the frequency of aberrations (Vig, 1978).

2.2. In Vivo Studies. Patients receiving chemotherapy have exhibited chromosome aberrations in bone marrow and lymphocytes. In the case of anthracyclines, studies with human subjects (Ballerini, 1970; Whang-Peng et al., 1969) have not only shown structural aberrations but also aneuploid cells have been recovered from subjects exposed to DNM (Ballerini, 1970; Sinkus and Orlova, 1970) and ADM (Massimo et al., 1972). Structural changes include all types of aberrations, such as rings, dicentrics and interarm intrachromosomal aberrations. Aberrations have also been observed in cells from patients given chemotherapy with ACM-D (Bridge and Melamed, 1972), MMC (Sinkus, 1972) and BLM (Bornstein et al., 1971). Bone marrow cells in rats expressed aberration rates 12–16 times higher than cultured leukocytes (Jensen and Philip, 1971), indicating differential sensitivity or wearing off of the effect of the drug. Leukemic cells grafted in mice show more sensitivity than nonleukemic cells (Stromskaya and Pogosianz, 1971).

In mice, MMC (Ehling, 1974; Epstein et al., 1972; Mitra and Malling, 1975) and ACM-D (Epstein et al., 1972) have been tested for induction of dominant lethals – a term referring to conglomerate of various types of aberrations. Such damage is absent when spermatozoa or late spermatids are treated (Malke, 1968), while early spermatids do express these mutations. Such dominant lethals have also been observed in *Habrobracon* (Smith, 1973). Mouse spermatocytes do not express any effect on visible chromosome aberrations, but a positive effect is observed in spermatogonia (Adler, 1974; Gillavod and Leonard, 1971). The effect in mice (Adler, 1973) and Chinese hamsters (Lavappa and Yerganian, 1972) has been attributed to the action of the drug on the S phase, with maximum effect in late-replicating constitutive heterochromatin (Adler, 1974). Bone marrow cells from Chinese hamsters treated with BLM express chromosome aberrations, as do micronuclei. In one study, ADM administered at therapeutic doses induced the formation of micronuclei, whereas BLM did not (Maier and Schmidt, 1976).

3. Sister Chromatid Exchange (SCE) Induction

The discovery that sister chromatids can be differentially stained with fluorescent dyes after BUrd incorporation for two cell cycles revolutionized the study of SCEs (Latt, 1974). Since then, fluorescence plus Giemsa-stained chromosomes have been tested for a variety of chemicals for the induction of SCEs. The genetic significance of such exchanges is unclear. However, the basic mechanism(s) involved in the production of SCEs are thought to be different from those involved in the production of chromosome aberrations (Sasaki, 1977). Several antibiotics have been found to increase their frequency when applied at doses far lower than those that barely induce traditional chromosome aberrations. BLM has been found to be effective in in vitro cultures of Chinese hamster (Perry and Evans, 1975), in human lymphocytes (Wolff et al., 1977), and in mouse (Allen and Latt, 1976; Lin and Alfi, 1976); DNM in mouse (Cornforth, unpublished data); and ADM in human lymphocytes and Chinese hamsters (Perry and Evans, 1975), to mention only a few examples. Kato's studies with MMC have indicated that excision repair (Kato and Shimada, 1975) might be involved in SCE formation. However, whereas Wolff et al. (1977) found that untreated xeroderma pigmentosum cells express SCE formation with efficiency as great as that of normal cells, MMC induced a higher frequency of SCEs in such cells than in normal ones.

A markedly less pronounced increase in the frequency of MMC-induced SCEs in patients suffering from Fanconi's anemia has been reported by Latt's group (Latt et al., 1955) in spite of a substantial increase in the frequency of traditional aberrations (Sasaki and Tonomura, 1973). However, patients with Bloom's syndrome exhibited a many-fold increase in the frequency of SCEs over the heterozygous or normal counterparts (German et al., 1971). Using data from MMC-induced SCEs, Comings has made a strong case for the involvement of constitutive heterochromation for observed similarities between production of SCEs, somatic recombination, and cross-configurations found in chromosomes in Bloom's syndrome (Comings, 1975). With MMC-induced SCEs, direct observations of somatic recombination (Huttner and Ruddle, 1976), involvement of constitutive heterochromatin (Lin and Alfi, 1976), and formation of hybrid DNA (Moore and Holiday, 1976) have been provided.

4. Effect on Recombination and Mosaicism

4.1. Meiotic. The data on the effect of antibiotics on meiotic recombination is limited to studies with MMC and ACM-D. MMC increases the frequency of recombination both in eu- and in prokaryotes, and the effect seems to be related to factors like DNA-synthesis period (Chiu and Hastings, 1973), germ cell stage treated (Suzuki, 1965b), and the genetic background of the organism (Dubnau et al., 1973). Examples can be cited from

Chlamydomonas reinhardii (Davies and Lawrence, 1967) and *Drosophila* (Schewe et al., 1971; Suzuki, 1965b) among eukaryotes. In *Paramecium caudatum* (Miwa and Hiwatashi, 1970), MMC has an indirect effect: it affects the mating ability, which might increase the chances of meiotic recombination in the population. Other effects include the transforming ability of DNA, e.g., increased fertility of the F population of *E. coli* K-12F and the induction of autonomous sex factors (Evenchik et al., 1969). On the other hand, MMC has been found to be incapable of inactivating transforming DNA, and may even lead to some block in the chain of events responsible for recombination, e.g., in *B. subtillus* (Cionini, 1973) and *Haemophilia influenza* (Kooistra and Venema, 1970).

Whereas in *Ornithogalum virens* (Church, 1973) no effect of ACM-D was found, an increase in the mean chiasma frequency has been reported in *Schistocerca gregaria* (Craig-Cameron, 1970), *Chlamydomonas* (Chiu and Hastings, 1973), and for the intervals *xc-an* (Dishler et al., 1975) and *trd-b* (Sinha and Helgason, 1969) in *Hordeum*. There appears to be some relationship between ACM-induced meiotic recombination and chromosome length (Craig-Cameron, 1970), genetic background of the material, i.e., inbred vs out-crossed strains (Craig-Cameron, 1970), treatment stage, e.g., in *Chlamydomonas* (Chiu and Hastings, 1973), chromosome region (Suzuki, 1963, 1965b), and DNA-synthetic activity of the cell (Chiu and Hastings, 1973).

4.2. Mitotic. The induction by BLM of red/pink twin sectors in the D7 strain of *Saccharomyces cerevisae* has been taken as evidence of the induction of mitotic crossing-over by this antibiotic (Hannan and Nasim, 1978). Similarly, BLM has been shown to cause gene conversion in this strain. However, of the antibiotics under consideration, MMC has been found to be the most potent inducer of somatic recombination, e.g., in *Ustilago* and *Saccharomyces* (Holliday, 1964), *Glycine max* (Vig and Paddock, 1968), *Arabidopsis thaliana* (Ahnstrom et al., 1972), and *Nicotiana tabaccum* (Carlson, 1974). Experiments with these organisms have helped establish, as originally postulated by Holliday (1964), the independence of mutagenic and recombinogenic effects. Additionally, Perry (1977) has suggested the induction of nondisjunction by MMC in *Saccharomyces*. In *Glycine*, the induction of somatic recombination, as determined by the production of twin sectors of two complementary homozygous phenotypes on the heterozygous background, is also accompanied by other genetic effects, as postulated by the production of single spots (Nilan and Vig, 1975; Vig, 1973a–c). The induced homozygosity has been correlated with the production of quadriradials by MMC in plant (Rajchert-Trzpil and Dobrzanski, 1968) and animal (Cohen and Shaw, 1964; German, 1964; German and LaRock 1964) chromosomes. The induction of mosaicism by MMC does not depend upon the application of the drug during the period of DNA synthesis (Vig and McFarlane, 1975) and is different from its induction by some alkylating agents, e.g., EMS (Vig et al., 1970). ACM-D also increases mitotic recombination in *Glycine*, even though with relatively low efficiency (Vig, 1973b).

When seeds of *Arabidopsis* are treated with MMC following treatment with methyl nitrosourea the plants exhibit chlorophyl-deficient sectors, which are attributed to a 'process similar to somatic recombination' (Ahnstrom et al., 1972). Similarily, mosaicism of an undefined nature has been observed in *Drosophila* (Walker and Williamson, 1975) and silkworm (Inagaki and Oster, 1969) after MMC treatment. Recently similar results have been obtained by Fahrig (1976) in inducing somatic sectors in mice treated with MMC during embryonic development. Of the anthracyclines, DNM has been found somewhat effective in inducing somatic crossing-over in yeast (Hannan and Nasim, 1979).

Carcinogenicity versus Mutagenicity

One of the factors limiting the application of antibiotic antineoplastic agents is their property to induce long-term toxicity, i.e., oncogenicity, mutagenicity, and teratogenicity (Marquardt et al., 1976). These effects are of serious concern not only in that induced mutations may be passed on to future generations, but also because some of these same mutagenic properties have been implicated in oncogenicity (Haddow et al., 1948; Knudson, 1973). Weinburger et al. (1975), Siebert et al. (1975), and, more recently, Marquardt and Marquardt (1977) have provided reviews regarding the tumorigenic properties of some antitumor agents.

Of the antitumor agents under discussion in the present review, the most effective ones, viz., the anthracyclines, have been shown to induce mammary and renal tumors in rats after the use of ADM (Philips et al., 1975) and after DNM (Sternberg et al., 1972). In a more recent study from the same laboratory (Marquardt et al., 1976), 6- to 7-week-old Sprague-Dawley female rats treated with 5 or 10 mg DNM/kg body wt. or 5 mg DNM/kg were shown to develop mammary fibroadenomas and carcinomas, and to a lesser extent adrenal carcinoma, epidermal carcinoma, liposarcoma, and Schwannoma. The induction of malignant transformation in M_2 fibroblasts of mouse origin has been suggested to be related to the production of 8-azaguanine-resistant mutant cell lines in V79 Chinese hamster cells (Marquardt et al., 1976) and to the production of base pair substitutions in TA 1535 mutants of *Salmonella* (Benedict et al., 1977b).

The intercalating antibiotic ACM-D, although similar to anthracyclines in its binding affinity to DNA, is almost ineffective in inducing tumors. This lack of activity has also been correlated with general lack of mutation induction by this chemical (Benedict et al., 1977b; Marquardt et al., 1976). However, tumors induced by ACM-D have been reported at least at the injection site (DiPaola, 1960), and might have been due to repeated and prolonged local injury and regenerative hypoplasia.

There are reports of in vivo induction of blastogenesis tumors by BLM (Llombart, 1976), and studies by Benedict et al. (1977a) have shown the capacity of this antibiotic for producing malignant transformation in mouse C3H/10T½ clone-8 cells. However, in spite of its chromosome-breaking action (see review by Vig and Lewis, 1978), BLM is only modestly effective in producing point mutations in yeast (Hannan and Nasim, 1978; Moore, 1978), and ineffective in the *Salmonella* test system (Benedict et al., 1977a and b).

The carcinogenic potential of MMC, if any, can be explained by two varying phenomena. First, the induction of point mutations, as discussed in the previous pages, may be responsible for triggering neoplastic growth. Secondly, the induction of mitotic crossing-over, which is so well achieved by the application of this antibiotic, could be responsible for expression of 'neoplasia-causing' recessive gene in an otherwise heterozygous cell population. Even though no direct evidence is available for such a relationship, there is ample evidence for the induction of reciprocal recombination induced by mitomycin C in somatic cells of mammals including man, of fungi, and of higher plants (see Vig, 1977, for review). The formation of DNA heteroduplexes by mitomycin D (Moore and Holliday, 1976), possibly involving constitutive heterochromatin (Comings, 1975), may explain the molecular mechanisms responsible for achieving homozygosity for such genes.

Conclusions

The preceding pages give an idea of the known genetic effects of some antitumor agents and the systems that have been developed and used to study such effects. However, from the point of view of mutagenesis and carcinogenesis the major task is to avert exposure to such agents. This, however, cannot be accomplished without the strong cooperation of state and federal agencies. Unquestionably, a multitude of chemicals to which we are exposed do not work singly or even additively. The greatest problem, apart from the incompleteness of our knowledge of the effects of various chemicals per se, is the lack of understanding of potentiation (or detoxification) of the chemical mutagens in interaction with other mutagenic or nonmutagenic agents in the system. This assessment of co-mutagenicity (Gebhart, 1971; Vig, 1978) appears to be crucial in decision-making processes, and there seems to be no rule of thumb applicable. For instance, ACM-D causes chromosome aberrations and induces somatic mosaicism, but may reduce the frequency of spontaneous mutations (Puglisi, 1968), X-ray induced mutations (Burdette, 1961; Proust et al., 1972), and translocations (Proust et al., 1972) on the one hand, while on the other, it may increase their frequency in interaction with other agents (Olivieri and Olivieri, 1965). Similar interactions have been observed between BLM and radiations (Byfield et al., 1976), BLM and ACM-D (Bearden and Haidle, 1975), MMC or BLM, and caffeine (Kihlman, 1960; Kihlman et al., 1974) and anthracyclines and nucleosides (Vig, 1973) or arginine (Vig et al., 1968b).

Another point of significance is the differential sensitivity of somatic vs germ cells. These data are not only available for experimental organisms, but in a few cases where mothers on chemotherapy have given birth to children, no damage has been found in the offspring (Cohen et al., 1969). Since the effect of chemicals wears off with time, the periods between the test exposure to drug and the time of reproduction must also be considered. However, this may not be critical for the carcinogenic effect of these antibiotics (Weisburger, 1977).

We must not also forget the arbitrary nature of the standards we set for ourselves. Geneticists normally talk in terms of a doubling dose. What is the rationale behind it? What do risk-benefit criteria mean? Are short-term tests infallible in predicting carcinogenicity and mutagenicity to eukaryotes, especially man? Above all, except in cases of drastically high exposure related to occupations or accidents, do we have enough data to claim that the human species shows induced heritable genetic damage? I have raised some of these questions not because they are new, but because I feel we must constantly keep sight of these shortcomings and look for ways to improve our information. Without question, once we let our genetic heritage 'deteriorate' we might be facing an insurmountable problem.

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