INTRODUCTION

On cytological grounds, 'nitrogen mustards' are halogenated alkylamines or substituted halogenated alkylamines vesicant in nature, can be classified as mitotic poisons(1). The nucleotoxic effects appear to be the most prominent in the heterochromatic regions and are best used as chemotherapeutic agents especially in the treatment of cancer.

The word 'Cancer' was used to describe any form of malignant tumor. The word has been taken from the Latin vocabulary 'Crab' which suggests the slow but inexorable spread of the neoplasms, clawing its way to other locations of the body. Cancer is a group of allied diseases, affecting the different organs and systems of the body, leading to the death of host, except in extremely rare cases of spontaneous regression. The cancer cells seem to proliferate freely in the host tissues, forming 'secondary deposits' or metastases and invading the normal tissues.

The normal mechanisms of growth control, the etiology of cancer and the manner in which it causes death is not understood, despite the effort that has been devoted to the studies of these phenomena.

It is generally assumed that cancer cells originate from normal cells by one or number of somatic mutations, or heritable alterations, which are formally equivalent to
a mutation, involving the disappearance of growth control. The mutation may be considered to be one by loss rather than by gain of a special device which confers autonomy to the cell. Among other reasons, upon the fact that every normal cell type is, either at some stage (postimitotics) or during the whole of its lifetime, endowed with a potential capacity to divide. The latent power of growth appears to be a general biological phenomenon. However, the normal cells are regulated by some homeostatic mechanism of unknown nature, probably of a complex repressive type, exercised by the organism as a whole. Any interference from outside which challenges the internal environment of control may release the latent growth, but as long as an irreversible change has not been induced by this interference the growth stops as soon as the original situation is being restored. In autonomous cancer cells, the suppressed growth potential is permanently liberated, whereas in hormone dependant tumors, growth is dependant on the endocrine imbalance which caused this original tumor cells to grow out. The latter cells may suddenly become autonomous, and the cell type - irrespective of its origin, tends to lose some of its biological characteristics. Finally, much of the changes observed during carcinogenesis appears to losses of properties and functions rather than development of new properties.
The common cancer-causing agents are believed to be present in the immediate personal environment of the individual acting through occupational hazards or from dietary and other habits and customs. Thus, men working in factories, where certain chemicals or intermediates are manufactured or employed, run the risk of having the suspect agent contaminate their skin or gain entry to their lungs, which many years later could give rise to cancer.

Cancer starts insidiously and does not give rise to any specific signs or symptoms at an early stage. However, its occurrence in a part of the body is often associated with the presence of one of the following seven warning signals depending upon the part that is affected:

(i) A sore that does not heal, particularly in the mouth.
(ii) Unusual and repeated bleeding, especially in women after menopause.
(iii) A lump or thickening in any part of the body, particularly the breast in women.
(iv) Persistent indigestion or difficulty in swallowing.
(v) Persistent hoarseness or cough.
(vi) A change in the usual bowel habits.
(vii) A change in the size and colour of a mole or wart.

The above mentioned signals do not always mean the presence of cancer.

A substantial number of investigators in Western
countries have shown that the major risk factors leading to the cancer of the uterine cervix are: early age at first marriage, at first coitus and at first pregnancy and include multiparity, a low socio-economic status, sexual promiscuity and co-habitation with uncircumsized male partners, etc.

The exact cause of the disease is not yet known. There probably is not any single cause but a combination of so-called contributory factors which ultimately lead to cancer. There is a lot of research being done on the subject (2). This is being carried out in three different directions - one to find the causative factors; the second - to diagnose the disease early and the third - to find better and safer methods of treatment.

The existing methods of cancer treatment could be divided into three major types. The different treatments which have been employed systematically are:-

(1) Treatment of cancer by radiotherapy.

(2) Treatment of cancer by surgery.

(3) Treatment of cancer by chemotherapy.

TREATMENT BY RADIOThERAPY

The role of modern radiotherapy in the cure and palliation of cancer has been reviewed by Tubiana and Chassagne (3). The treatment is based on the principle
that a high energy radiations of the desired dose are introduced into the selected tissues and the tolerance is being improved because the integral dose, i.e., the total dose received by the organism during the irradiation of the tumor, is usually less than the expected dose. In particular, the hematological disturbances associated with the radiation are less pronounced. Local or general intolerance, however, although troublesome, tended to protect the patient from over dosage. As it has become possible to deliver almost unlimited doses to the body, it has become necessary to measure with the greatest possible accuracy the quantity of radiations given, to establish the optimal dose for each tumor and to deliver exactly this dose to the chosen tissue.

Dosimetric techniques are employed to measure the quantity of radiation absorbed by the tumor and the neighbouring tissues, especially tissues those are radiosensitive. The increased accuracy of dosimetry is a major factor in the progress of radiotherapy. The margin for manoeuvre between underdosage and overdosage is often narrow and the optimal dose varies from one system to other. This can only be established by statistical methods using therapeutic trials.

Radiotherapy still has considerable prospects. The introduction of high-energy protons and then negative
mesons may be regarded as offering the possibility of a more exact and powerful effect on tumor cells, with minimal damage to neighbouring healthy tissues. The new techniques may improve the results of radiotherapy of deep-seated tumors, as of the lungs or esophagus. Radioactive isotopes also have a part to play in the treatment of certain tumors.

TREATMENT BY SURGERY

The modern state of surgery and anesthesiology is based on the principles, to permit operative removal of tumors of any location. The aims of the surgical treatment of cancer are twofold: firstly, 'curative', i.e., to eradicate the disease; or secondly, 'palliative', i.e., to relieve pain and distress, and may be, to prolong life.

The essential feature of curative cancer surgery is that it should be adequate. To be so, the growth and its local surroundings must be completely removed, with a safety margin of uninvolved normal tissues. It may entail the removal of a whole organ such as the breast or the thyroid gland, the stomach or the kidney; or it may demand the sacrifice of a limb, as in some forms of malignancy in bone. In superficial and observable cancers it is generally easy to select the potentially curable ones. In case of internal tumors the decision for or against radical operation, can be made only when the tumor...
is displayed at operation and its possible branching and division of parts investigated.

When the cancer has advanced to a stage at which cure is impossible, or the disease is irremovable, or when it has recurred, further treatment is 'palliative'. This implies that it is intended to relieve, as far as possible, the distressing and disabling sequelae of the disease. In this human exercise surgery has often an important part to play, some times alone, some times in concert with irradiation, hormones or the use of anti-cancer drugs. The development of transplantation surgery opens up new fields for surgical oncology(14).

The surgical treatment of cancer is somewhere near its limits. More often unsatisfactory results are due not to the lack of possibilities in modern surgery but to the inadequate results attained in the course of treatment. The reason for this lies in the disease itself, its obscure pattern, peculiarities, and capacity for producing metastases and becoming generalized. Even the greatest success of transplantation surgery will not change this situation; these will be significant to a few patients only.

TREATMENT BY CHEMOTHERAPY

Another method of cancer treatment is of special
interest in our days — the administration of chemotherapeutic compounds and hormones. It is fully justified to regard antitumor chemotherapy as feasible, on the ground that chemotherapy means the selective inhibitory effect of a drug on tumor cells. The term itself was introduced by Ehrlich into the medical practice to define a mean of exerting such as selective influence on pathogenes. The question of its suitability for application to tumors is, therefore, undisputable.

The cancer chemotherapy dates from 1946, when the first clinical reports on the therapeutic effects of urethan(5) in myeloid leukemia and the study of the effect of nitrogen mustard in Hodgkin’s disease, chronic leukemia and lymphoreticulosarcoma was published by Haddow et al.(6). During the same time the existence of Hormones(7) came into the field for the treatment of cancer.

During the short period of about thirty years nearly fifty anti-cancer drugs, that are used with some effect in many forms of malignant tumors, have been developed. With the better understanding of the nature of cell replication it is now possible to device compounds of potential usefulness and with the better understanding of the subject, more effective compounds shall be developed in the near future.
The existing agents may be divided into the following main groups:

(a) Antimetabolites.
(b) Antibiotic-type substances.
(c) Substances from Plant Origin and Alkaloids.
(d) Hormones and related compounds.
(e) Other compounds.
(f) Alkylating agents.

Agents of all these groups have been developed simultaneously. Thus in 1948, two years after the introduction of nitrogen mustards and urethan derivatives the therapeutic properties of the first anti-metabolite was discovered. This was an analog of folic acid, aminopterin (Ia) (8). Somewhat later methotrexate (amethopterin) (Ib) was discovered (9).

A large number of compounds were developed and their effectiveness was established in 1954-55 (10, 11a, 12). An impressively detailed and persuasive report on the different types of existing findings has recently been published by Schmidt et al. (11b).

(a) Antimetabolites

An antimetabolite is, by definition, a substance which interferes with the formation or function of a natural metabolite. Such substances appear commonly to
attack by combining with a specific enzyme site, thereby preventing access of the true substrate to the site. In general, metabolite and anti-metabolite are structurally similar, although molecular likeness does not guarantee biological activity. There are many cases where quite small structural changes reduce or abolish the activity of an antagonist or a slight modification may give a more active compound. Tumor inhibition is often greater when two agents are introduced simultaneously rather than a single agent. No specific structure-activity relationship could be worked out and only the experimental results provide the safe grounds. Various aspects of antitumor drug synergism have been discussed recently by Venditti and Goldin(1964)(13).

The discovery of remission in children with acute leukemia, by aminopterin(Ia) was initiated by Farber and his colleagues(9) and the field was then further reviewed and worked out by a large number of workers(14). Among them Calabresi and Welch(1962) (15) gave a comprehensive review in this field. The general compound of basic interests are:

(Ia) $R = H, \ X = X' = H \quad (\text{aminopterin})$
The other effective halogenated derivatives were reviewed by Goldin et al. (1959) (16). The aspartic acid analogs of aminopterin, 4-aminoteroylaspartic acids had widely examined by Hitchings et al. (17). The various series of the compounds which have been thoroughly dealt with are mercaptopurines, found effective against human cancer, was prepared on the semirational basis (18) and was found to produce temporary regression in case of acute leukemia. Thioguanines (19), azaguanines (20), fluoropyrimidines (21) and other ribosides (22), azauracils and azauridines (23) and aminocyclopentane carboxylic acids (ACP) etc. are the other important compounds of this family. Amino acid antagonists (24), Folic acid antagonists (11), Septacidin and adenine derivatives in which sugar moiety is attached are the most commonly used agents for the treatment of various types of tumors.

There are indications, however, that the action of the folic acid antagonists is directed against all rapidly proliferating cells and not specifically against neoplasms (26). The derivatives of folic acid are extremely toxic because they prevent a variety of metabolic transfer of one-carbon units, which is essential for the life and
reproduction of cells. One of the most important folic acid derivative is \(N^{10}\)-formyl 5,6,7,8-tetrahydrofolic acid (f\(^{10}\)FH\(_4\)), which acts as formyl donor(27).

In case of pyrimidine analogs, DDMP(II), has been found as one of the most effective agent as feedback inhibitor(28). Similarly dinucleotide derivatives of purine(III) have been found suitable as inhibitors.

(b) **Antibiotics**

Antibiotics have been defined as the chemical substances produced by micro-organisms having the capacity to inhibit the growth of other organisms in dilute solutions.

Actinomycines and related compounds have been reported in 1940(29). These materials are powerful bacteriostatic agents and are extremely toxic(30). Their
cytostatic properties aroused interest in their potential anticancer activity (31).

All the actinomycins (IV) contain the same 2-amino-4,6-dimethylphenoxaz-3-one-4,5-dicarboxylic acid chromophore with variety of polypeptide side chains attached via carboxylic functions. The lactone rings are required for biological activity because the free chromophore is not active. Similar is the case with polypeptide chains. Actinomycine C4 is one of the most important member of the series and has been widely used against various animal test systems. It is useful clinically, but its utility is limited because of greater toxicity.

In addition to the actinomycine, several other
antibiotics with antitumor activity, including the anthra-
cydines - daunomycin (rubidomyein), cinerubin, nogalamycin
and the chromomycins- including mithramycin and olivomycin,
are known to form complexes with DNA, in different ways but
with the same end effect - blocking synthesis of RNA and of
proteins (32).

The other important antibiotics are actidiones,
puromycin, mitomycin C, sarkomycin (33), fumagillin (34),
streptonigrin (35), tenuazonic acid and actinogain isolated
from natural glycopeptides (36, 37).

(c) Substances from Plant Origin and Alkaloids

Basically the plants have been found to be a good
source of many important drugs but the early work on plant
products as potential anticancerous agents was not encoura-
ging. Colchicine (V) (38), an alkaloid isolated from plant
colchicum-autumnale inhibits cellular mitosis in metaphase
by dis-orienting the structural organisation of the asters
and the spindle (39). No authentic mechanism of its action
could be worked out as yet. Podophyllotoxin (VI) extracted
from the plant podophyllum peltatum resembles colchicine
in many respects but no good results could be achieved
from the use of either of them.
Vinca alkaloids: The extracts of periwinkle plant, vinca rosea Linn, were originally studied because of the reports that the tea made from the leaves of the plant was useful in the control of diabetes mellitus. Although no alterations in blood sugar level in treated normal or diabetic animals were in fact observed, it was found that injection of the extracts into rats resulted in the death of the animals from septicemia. This observation led directly to the discovery of the leukopenic and carcinolytic effect of the extracts. The biological and chemical properties of the vinca alkaloids have been reviewed in detail by a large number of workers. More than 30 alkaloids have been isolated from extracts of vinca rosea and four of them showed substantial antitumor activity. These are vinblastine (vincaleukoblastine, VLB) (VIIa), vinleurosin (leurosine), vincristine (leurocristine) (VIIb) and vinrosidine (leurosidine) (VIIc). The structure of vinblastine and vincristine
have been completely elucidated [42]. Vinblastine have been used in combination with chloroambucil in the treatment of Hodgkin's disease and of ovarian carcinoma. The activity of vinblastine could not be confirmed against leukemia L1210 in mice as shown by Cutts et al. (1960) [43], but recognized that differences in animal strain may have accounted for the disagreement. The structure of the vinblastine and vincristine could be represented as:

(VIIa) Vinblastine \( R = \text{CH}_3 \)

(VIIb) Vincristine \( R = O = \text{C} - \text{H} \)

These alkaloids are similar chemically. They are asymmetrical dimeric compounds. Complete remission has been seen in some cases of acute monocytic leukemia and of mycosis fungoides with vinblastine.
Aisenberg and Wilke's (1964) have demonstrated that vinblastine and vincristine are potent immuno-suppressive agents in the rats. Antibody formation and delayed hypersensitive to bovine serum albumine and hypersensitive to the tuberculin were suppressed. Inflammatory responses were reduced and homograft rejection was delayed. No marked depression of lymphocytes in the peripheral blood was seen during immuno-suppression with vincristine.

(d) Hormones and related compounds

This class of compounds is widely distributed in the plant and animal kingdoms and includes sterols, the bile acids, the cardiac active aglycones, the sapogenins, the steroidal alkaloids, provitamin D₃, which leads to vitamin D₃ and certain other hormones. Steroids are frequently associated with vital biological functions, this being especially true of the compounds which are to be considered. The word "Hormone" (from Greek word 'hormao' means excite or arouse) was introduced by Starling (1905) for those internal secretions into the blood stream which act as "chemical messengers" and exert their effect at a point removed from their source.

Hormonal agents were firstly used against cancer of breast and prostate. Hormonal therapy is based on the concept that neoplastic cells derived from a
hormone-responsive organ may likewise be subjected to hormonal control, at least during some part of the affected side as explained earlier. Nevertheless, the fact that some cancer cells are not completely autonomous, but are subject to restraining factors (hormones) similar to those operative for normal cells, is of fundamental importance to the cancer problem.

The compounds that sometimes evoke the remission of a malignancy, belonging to hormone-responsive family are not necessarily confined to one of the traditional classes of steroid hormones (estrogens, androgens, progestogens, corticosteroids). Further to this the proliferation of structurally modified steroids during the past few years has resulted in a blurring of both the structural and the physiological lines of distinctive among those traditional classes. For example, fluorometholone (VIII) or its acetate, which is active against more than one kind of neoplasia is potent, as both a progestational and a corticosteroid (glycogenic and anti-inflammatory agent) (49). The
chemotherapy studies of hormone-responsive malignancies have been hampered because of the dearth of experimental animal neoplasms that stimulate the human diseases. The work has been reviewed by various workers (50-52).

(e) Other compounds

Methylhydrazine derivatives: A new type of antitumor agent emerged from a search among substituted hydrazines for monoamine oxidase inhibitors (53). The work has been summarized by Bollag (54). All the experimentally effective antitumor compounds contain the methyl hydrazine residue, CH\(_3\)NH·NH·, and the greatest activity was shown by those in which this group is attached to a substituted benzyl radical (46). The crystalline salts of the hydrazine were stable, but their solutions tended to oxidize, especially where water was the solvent.

One member of the series N-isopropyl-α-(2-methylhydrazino)-p-toluamide hydrochloride (IX), is used clinically.

\[
\begin{align*}
\text{CH}_3\text{NH}·\text{NHCH}_2 & \quad \text{CONH}·\text{CH(CH}_3)_2·\text{HCl} \\
\text{(IX)}
\end{align*}
\]

Like other antitumor drugs, the methylhydrazines do not act specifically on tumor tissues. They exert a
suppressive effect on the hemopoietic system, and mainly affect the lymphoid and myeloid cells. There is no close correlation, however, between bone marrow depression and antitumor activity. Many of the hydrazine derivatives also caused hemolysis, and Heinz-Ehrlich inclusion bodies were seen in the erythrocytes. Secondary effects of the hemolysis were reticulocytosis and the storage of hemosiderin in various organs. All the methylhydrazines markedly depressed spermatogenesis, and led to testicular atrophy.

**Methylglyoxal-bisguanyl-hydrazone (Methyl -GAG)**: The carcinostatic action of various hydrazine derivatives led Freedlander and French (1958) (55) to examine a number of hydrazones, including glyoxalbisguanylhydrazone (GAG) (X) and its methyl derivative. These compounds inhibit experimental tumors but Me-GAG was rather more active than GAG and has been subject of more intensive investigation. Recent reviewers includes White, Milich and Montgomery (56, 32).

\[
\begin{align*}
\text{CH}_3\text{C} &= \text{N.NH}_2\text{C}.\text{NH}_2 \\
\text{H}_2\text{C} &= \text{N.NH}_2\text{C}.\text{NH}_2 \\
\text{NH} &= \text{NH}
\end{align*}
\]

(X)

**Phthalanilides**: The properties and antileukemic effects of several substituted anilides of terephthalic acid have
been recently reported. The striking activity against leukemia L 1210 in mice was confirmed in a number of studies published simultaneously. The active compound of this family have certain features in common (a) strongly basic in nature, sterically little-hindered groups attached to benzene rings; and (b) bridging groups of the amide or urea type; when the link was \(-\text{NiCH-} (\text{Azomethine})\) or \(-\text{NH.SO}_2-\) (Sulfonamide)\(^{(57)}\).

\[
\text{C}_6\text{H}_4\text{COHN-}\text{C}_6\text{H}_4\text{CONH-C}_6\text{H}_4
\]

(UXI)

**Urethan and its derivatives:** Urethan (ethyl carbamate)\(^{(XII)}\), although chemically simple, shows many diverse biological effects. The polydynamic nature of the agent has been screened in detail by Haddow\(^{(58)}\). The compound was found active in suppressing the growth of the Walker carcinosarcoma 256 in rats, and other experimental tumors have since been found to respond:

\[
\text{H}_2\text{N.CO.OC}_2\text{H}_5
\]

(XII)

**Styrylquinolines:** Haddow et al.\(^{(59)}\) reported the activity of a number of styrylquinolines\(^{(XIII)}\) against the Walker rat carcinosarcoma 256. A few years later American workers observed that their salts as methyliodides or methyl chlorides, given orally with the diet, caused
complete regression of the established lymphoma 8 rat tumor. The general compounds belonging to this class are represented as:

\[ \text{Hydroxyurea} : \text{Hydroxyurea (XIV), prepared as one of a number of hydroxylamine derivatives, was found to have activity against leukemia L1210 in mice, including strains resistant to methotrexate, thioguanine and 8-azaguanine.} \]

Prolonged oral or intravenous administration of the compound in dogs and rats produced hypoplasia of the bone marrow, lymphoid depletion and some spermatogenic arrest. The compound was teratogenic in the rats, chick and sand dollar embryo(60). Bone marrow depression and gastrointestinal toxicity from repeated doses were referred to in as earlier report, which also mentioned the sedative and neurological effects in mice, rats etc.

\[ \text{H}_2\text{H}.\text{CONH}.\text{OH} \]

\[ \text{(XIV)} \]
1,3-Bis-2-Chloroethyl-l-nitrosourea (BCNU): BCNU (XV) (61), formally a nitrogen mustard, was the most effective of a series of nitrosoureas against intracerebral and subcutaneous L 1210 mouse leukemia. It also increased the survival time of mice with advanced Moloney leukemia LST-R-A. Venditti et al. (1965) (62), reported that the BCNU in combination with the methotrexate was more effective against advanced L 1210.

\[
\text{NO} \quad \text{Cl}_2\text{CH}_2\text{CH}_2\text{N.CO.NH}_2\text{CH}_2\text{CH}_2\text{Cl} \\
\text{ClCH}_2\text{CH}_2\text{N.CO.NH}_2\text{CH}_2\text{CH}_2\text{Cl}
\]

(XV)

Kethoxal Bis-thiosemicarbazone (KTS) Methylglyoxal Bis-N-methyl-thiosemicarbazone (MGMS) and related compounds: KTS (XVI) and MGMS (XVII) were among the most active of a number of \(\alpha\)-Ketoaldehyde bis-thiosemicarbazones against experimental tumors. KTS inhibited sarcoma 180 and adenocarcinoma 755 in mice and Walker Carcinoma 256 in rats. MGMS also showed the similar antitumor effects (63).
Purin-6-yltrimethylammonium chloride (XVIII): This quaternary ammonium salt was active against the Ehrlich ascites tumor and epidermoid carcinoma DC 5. No significant activity was seen against sarcoma 180 or leukemia L 1210 and other tumors (64).

\[
\text{(XVIII)}
\]

4-Nitroquinoline-N-oxide: Sakai et al. (1955) (65) reported the effect of number of quinoline derivatives against the solid and ascites forms of Ehrlich carcinoma in mice. On the most active was 4-nitroquinoline-N-oxide (XIX). Other investigators have since confirmed the antitumor activity of the compound in vivo in several systems, but Moore et al. (1960) (66) concluded that the tumor inhibition was probably due to the general toxic effects of the drug.

\[
\text{(XIX)}
\]

Thiocarzolamide: Thiocarzolamide (XX) was active against adenocarcinoma 755 and spontaneous mammary tumors in mice.

\[
\text{(XX)}
\]
Miracil D (Leucanthone): Some years ago, in a search among analogs of the antimalarial drug quinaerine, Hirschberg et al. (1959) (67) showed that the 10-thiaxanthenone derivative, Miracil D (XXI), was active against a wide spectrum of transplantable tumors at non-toxic doses. Later on it was found that the compound was more effective against methylcholanthrene induced rat mammary carcinomas.

![Chemical Structure of Miracil D](image)

Cucurbitacins: The cucurbitacins (XXII) are a group of highly oxygenated triterpenes from various species of the cucurbitaceae plant family. The structure of these compounds is fairly established and four of the compounds showed moderate antitumor activity (68).

![Chemical Structure of Cucurbitacins](image)

Aristolochic acid: Extracts of the plant aristolochia indica have been used in the treatment of cancer. Recent
studies demonstrated activity of such extracts against adenocarcinoma 755 in mice.

The compound had no significant activity against sarcoma 180 in mice or sarcoma M1 or 45 or carcinoma PC I in rats.

(f) Alkylating agents

The biological effects of alkylating agents as a class of chemically reactive compounds was made by Ehrlich, when he noted the structural analogy between ethylene oxide and ethyleneimine and studied their unusual pathological changes occurring in animals after administration of the lethal doses of the later(69). Meyer had earlier noted the poisonous and vasicant effects of sulphur mustards on rabbits(70). In the interval between World War I and II, extensive studies of the biological and chemical actions of nitrogen mustards were conducted. Modern interest in chemotherapeutically useful alkylating agents, however, dates from the discovery of the carcinolytic activity of nitrogen mustard as revealed in a classic summary of work.
on the biological effects and therapeutic applications of certain chemical warfare agents during the IIInd World War. Since that time a large number of chemically reactive substances classified as alkylating agents have been synthesised for the purpose of antineoplastic evaluation in various test systems and some of them have shown good results in the treatment of various types of cancer.

Several reviews have been written on these agents that relate to their mechanism of action, general pharmacology and therapeutic applications. The previous reviewers which helped in bringing the whole literature to one stand are Brookes and Lawley (1964) (72); Brown (1963) (73); Philips (1950) (74); Ross (1953, 1962) (75, 76); Karnofsky (1958) (77); Mandel (1959) (78); Emmelot (1965) (79); Montgomery (1959) (80); Wheeler (1962, 1969) (81, 82); Warwick (1965) (84); Skinner et al. (1960) (83); Hirschberg (1963 (85); Calabresi et al. (1966) (86); Farber et al. (1956) (87); Bratzel et al. (1963) (71) and many others.

The simplest bifunctional nitrogen mustard, methyl-bis(2-chloroethyl)amine or HN2 (XXIV) (76), is one of the most widely known prototype compound in which methyl group can be easily replaced by simpler or more complex substituents without any loss in the carcinostatic activity or

\[
\text{CH}_3\text{N(CH}_2\text{CH}_2\text{Cl)}_2
\]

(XXIV)
significant alteration in the therapeutic index. Mustergen and the other highly reactive nitrogen mustards are toxic, vesicant, unstable compounds that are best handled as the crystalline, water soluble hydrochloride salts. At physiological pH aliphatic nitrogen mustard hydrochlorides such as HN2 are readily and unimolecularly connected via the free base to a relatively stable aziridinium ion, which in turn reacts bimolecularly with available nucleophilic centres. The second arm of HN2 and similar bifunctional agents may then react by the repetition of this cyclisation process as explained above. The simplest nitrogen mustard, nor HN2 at a particular pH value, is not basic enough to exist as a stable cation.

In the similar investigations the tetrafunctional bridged mustard was found no more effective than the HN2, while monofunctional 2-chloro-N₂N-dimethylethylamine was inactive. Other type of mono substituted nitrogen mustard compounds (XXV and XXVI) also failed to show any anticancer activity in various animal test systems (91).

\[(\text{ClCH}_2\text{CH}_2)_2\text{N.CH}_2\text{.CH}_2\text{.N(CH}_2\text{CH}_2\text{Cl})_2\]

(XXV)
In a series of aliphatic nitrogen mustards of the general formula (XXVII), in which the alkylating moiety, -N(CH₂CH₂Cl)₂, appears in increasing profusion, were first examined at the Sloan-Kettering Institute, but they offered no improvements over HN₂. The most promising chemotherapeutic indices against Yoshida Sarcoma belonged to n = 2, m = 6 and 9 (92).

\[
\begin{align*}
\text{R. N. (CH₂)ₙ Cl} \\
\text{(CH₂)m} \\
\text{R. N. (CH₂)ₙ Cl}
\end{align*}
\]

(XXVII)

(XXVIIa) n = 2, m = 3 and R = CH₃

(XXVIIb) n = 2, m = 3 and R = C₆H₅⁻

The general structure with R = CH₃ (XXVIIa) were found inactive against a number of animal tumors (93), but m = 3-5 were active against mouse Leukemia L 1210 and either inactive or negligibly active against mouse sarcoma 180 and Walker rat carcinosarcoma 256 (94).

When R = C₆H₅ (XXVIIb), in a series of aromatic nitrogen mustards, the activity was found to be restricted
to those compounds in which \( m = n = 2 \) and \( m = 3, n = 2 \), activity decreased when \( m > 3 \), and as a result of diminished chemical reactivity (95).

Replacement of the methyl group of HN2 by groups that markedly alter basicity affects both chemical reactivity and biological activity, as rendered by a study of the aromatic nitrogen mustards of general formula:

\[
(C_1CH_2CH_2)_2N-\text{[structure]}-R
\]

(XXVIII)

(XXVIIIa) \( R = -H, -\text{CH}_3, -\text{OCH}_3, -\text{NH}_2, -\text{Cl}, -\text{NO}_2, -\text{CONH}_2, -\text{CHO} \).

(XXVIIIb) \( R = (\text{CH}_2)_n\text{COOH}; n = 3 \) (chlorambucil)

(XXVIIIc) \( R = 0(\text{CH}_2)_n\text{COOH} \).

In this series \(-N(\text{CH}_2\text{CH}_2\text{Cl})_2 \) is attached directly to the benzene ring, was developed largely through the efforts of the Chester Beatty Cancer Research Institute group led by Sir Haddow and the Russian investigators associated with L.F. Larionov. The less reactive mustards apparently do not alkylate under physiological conditions, relating anticancer activity to the minimal degree of chemical reactivity. Electron-donating substituents on the phenyl group increase reactivity of the mustard moiety, whereas electron-withdrawing substituents have shown to
react by SN\textsuperscript{1} mechanism\textsuperscript{(96)}, but subsequent evidence, based on kinetic studies, indicates that chlorambucil(XXVIIIb) and sarcolysin-1(XXIX) react via a two step process involving a formation of a relatively stable intermediate similar as in case of aliphatic nitrogen mustards\textsuperscript{(97)}. A three membered ring intermediate was suggested to be formed by the aromatic nitrogen mustard\textsuperscript{(98)}. The majority of nitrogen mustards studied are characterised by bis(2-chloroethyl) amino group, i.e., -N(CH\textsubscript{2}CH\textsubscript{2}Cl)\textsubscript{2} moiety, but favourable changes in chemical reactivity and biological activity have sometimes been affected by varying the halogens. For example, the antitumor activity of p-(bis(2-bromoethyl) amino) benzenethiol(XXX) was reportedly superior to that of the moderately active chloro analog\textsuperscript{(99)}.

\[
\text{N},\text{N}-\text{Bis(2-bromoethyl) amino sulfanilamide(XXXI)},
\]

one of the three bromomustards in a series of alkylating sulphonamides that had shown antitumor activity\textsuperscript{(100)}, produced complete and permanent regression of Murphy-Sturn
lymphosarcoma and Walker carcinosarcoma 256(101).

\[(\text{BrCH}_2\text{CH}_2)_2\text{N-} \begin{array}{c}
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\end{array}\text{-SO}_2\text{NH}_2.\]

(XXXIX)

The monofunctional N-(2-bromoethyl)-N-ethyl sulphanilamide was even more potent against Murphy-Sturn tumor than the bifunctional analog - an observation constructed to support the suggestion that bifunctionality may not be a prerequisite for anti-tumor activity in alkylating agents that act at a specific cellular site other than DNA(102). In case of iodo-substituted compounds the anticancer activity was found to be negligible, may be because of the complexity of the reaction mechanism(76).

With respect to activity against Walker Carcinosarcoma 256, a favourable comparison of the dibromo mustard and the mixed bromo chloro mustard with a highly active dichloro mustard has been reported(103).

```
(XXXIIa) X = Y = Cl
(XXXIIb) X = Br, Y = Cl
(XXXIIc) X = Y = Cl

The activity was found to be as X = Y = Cl \( \triangleright \) X = Br,
Y = Cl, X = Y = Br. In case of 2-fluorethylamines and other fluoro compounds were found to have negligible reactivity. This low order of chemical reactivity, however, would appear to make the fluoro mustards ineffective as anticancer agents. The mixed chlorofluoro mustard, florpan (XXXIII) has shown clinical activity, a remarkable complete remission of osteogenic sarcoma (104).

\[ \text{XXXIII} \]

The replacement of one or both chlorine atoms of chlorambucil (XXIIb) and the other related compounds by fluorine, resulted in the increase of toxicity and inactivity against various animal tumors (105).

Chlorambucil (XXIIb) (106) and the isosteric phenoxy propionic acid analogs (XXIIIc) (107) produced exceptionally high activities against Walker Carcinoma 256, among the number of other related compounds. This could not be related to reactivity differences and were attributed to the transport characteristics of the groups to which the mustard group is attached (76). A large number of compounds based on this principle, have been synthesised with comparable activity against Walker Carcinoma 256 (11b, 108). Such a concept has led to the search for structural
modifications that would enhance selectivity for cancer cells and mitigate toxicity. Early work on active transport on naturally occurring aminoacids resulted into the synthesis of highly reactive L-phenylalanine mustard (109). The high activity of the DL-form (sarcolysin (XXIX) (110)) has been attributed to the presence of L-isomer, although, L- and D- isomers were not significantly different in the various transplantable tumor cells and there were only an approximately two-fold difference in potency (111). The clinical utility of sarcolysin derivatives prompted the synthesis of many related compounds (112), including the typtophan (XXXIV) and their homologs (113).

![XXXIV](attachment:image)

Peptide derivatives (114) such as ω-g-lutamyl metphalan ethyl ester, whose L, L-diastereomer proved to be more active against various tumor systems (115), than the DL-diastereomer, though more toxic (116).

![XXXV](attachment:image)

Cholesteryl esters (XXXVI) (117) have also been found effective against Walker rat carcinosarcoma 256.
Similarly other peptides like leucine, methionine and hydantoin showed activity against various tumor test systems (98). Indan carboxylic acid (XXXVII) has been found against breast cancer (118) in number of biochemical tests.

Different type of amino acid mustards in which the amino group has been converted to the alkylating function have also shown activity against both experimental animal and human cancer. N,N-Bis(2-chloroethyl) glycine (XXXVIIa) and N,N-bis(2-chloroethyl) alanine (XXXVIIb) are the well known compounds of this family (119). The compounds could be represented as:

\[
\begin{align*}
N(\text{CH}_2\text{CH}_2\text{Cl})_2 & \\
R-\text{CH}_2\text{COOH}. & \quad \text{(XXXVIII)}
\end{align*}
\]

(XXXVIIa) \( R = \text{H} \); (XXXVIIb) \( R = \text{CH}_3 \)
Another highly active example of stereospecificity due to a carrier group is found in the series of degranol, mannitol, mannomustine compounds in comparison to that of the 1,6-bis(2-chloroethyl)amino-1,6-dideoxy-D-mannitol (120). The isomeric dulcitol analogs (93) have been found inactive.

\[
\begin{align*}
&\text{CH}_2\cdot\text{NH}(\text{CH}_2)_n\cdot\text{X} \\
&\text{(CHOH)}_m \\
&\text{CH}_2\cdot\text{NH}(\text{CH}_2)_n\cdot\text{X}.
\end{align*}
\]

(XXXIX)

(XXXIXa) \( n = 2, \ m = 4, \ X = \text{Cl} \)

(XXXIXb) \( n = 2, \ m = 4, \ X = \text{Br} \).

The dibromo analog of mannitol (XXXIXb) (119) was found to be more effective against several transplanted rat and mouse tumors, than its dichloro counterpart (XXXIXa) (120). Recently it has been reported that the derivatives of dulcitol mustard, have shown pronounced inhibition of various animal tumors (121).

\[
\begin{align*}
&\text{CH}_2\text{Br} \\
&\text{(CHOH)}_4 \\
&\text{CH}_2\text{Br} \\
&\text{(XL)}
\end{align*}
\]

Carbohydrates have been efficiently incorporated.
into other nitrogen mustards, e.g., 1,4-bis(2-chloroethylamino)-1,4-dideoxyerythritol (XLI), and monofunctional and bifunctional nitrogen mustard analogs of D-glucose and D-ribose (121). Two monofunctional derivatives of D-glucose (R = CH3 or R = C6H5) (XLI or XLII) showed considerable activity against leukemia L1210, in contrast to the borderline activity to the bifunctional derivatives. Both types of D-ribose were also found active.

\[
\text{(XLI)}
\]

\[
\text{(XLII)}
\]

The structure of the reported thiocytosine analog of nitrogen mustard (XLIII) was also shown to have the monofunctional cyclized structure, having low toxicity (122, 123).

\[
\text{(XLIII)}
\]

Intramolecular cyclisation, resulting in the diionic structure, with very low toxicity, was encountered with cytidine mustard (124). Earlier thymine and thymine
ribonucleoside mustards were reported, the later being the first example of nucleoside mustard (XLV)

\[
\begin{align*}
\text{(XLIV)} \\
\text{(XLV)}
\end{align*}
\]

The unique combination of uridine and benzaldehyde mustard synthesised by Belikova et al. (125) showed good activity. The thymine mustard 5-bis(2-chloroethyl) aminomethyl-uracil (XLIV) was reported effective against mouse leukemia (126).

Another series of the compounds belonging to pyrimidine (XLIV) and uracil (XLV) derivatives, both exhibited clinical activity. Dopan (XLVI) was one of the most important pyrimidine derivative (127).

\[
\begin{align*}
\text{(XLVI)}
\end{align*}
\]

The adenine mustards, in which the bis(2-chloroethyl) amino-group is not attached directly to the heterocyclic ring, are not subjected to intramolecular cyclization (128). 8-Bis(2-chloroethyl) amino-substituted purines,
such as adenine, cyclize readily at N-7 or N-9 of the purine ring, as does an analogous purine sulphur mustard derivative (129), has apparently not been established.

\[
\begin{align*}
\text{(XLVII)} & \quad \text{(XLVIII)} \\
\end{align*}
\]

A combination of 6-mercaptopurine and nitrogen mustard was achieved in the synthesis of 6-((2-bis(2-chloroethyl)amino)ethyl thio)purine dihydrochloride (XLIX) (130), whose activity resembles more closely to that of HN2 than that of 6-mercaptopurine (131).

\[
\begin{align*}
\text{(XLIX)} \\
\end{align*}
\]

Other representative heterocyclic compounds are the pyridone (XLV) derivatives (132) and benzimidazole mustards (133). The surprising degree of antitumor activity have been shown by the monofunctional 5-bromo-3-((2-chloroethyl)ethylamino methyl)benzophenone hydrochloride (LII) in comparison to that of monofunctional sulphanilamide derivatives (134).
Another series of the compounds was established in vivo by reductive cleavage of azo compounds to amines.

\[
\text{BCO.\text{NHC}H_2\text{Cl}2} \quad \text{(LII1)}
\]

These amides, however, limits the clinical utility of the various compounds. The rearrangement on the other hand, become a synthetic asset as the basis of a convenient preparation of mixed bromochloro-mustards(LIV)(100).

\[
\text{CH}_3\text{N}^+\text{CH}_2\text{Cl} \quad \text{(LIV)}
\]

The reported localisation of tetracycline in both animal and human neoplastic tissues inspired an investigation of a tetracycline-based nitrogen-mustard, which are rationally assigned the structure(LV)(135). The properties of activity against Adenocarcinoma 755 and Leukemia L 1210 with the greater effectiveness of aged solutions and the similarity of its dose response to that of HN2, suggest an in vivo cleavage at the amide bond and release of an HN2 like nitrogen mustard(136).
The stable aromatic triazenes derived from the fluoro homologs of N or HN2(LVI), hydrolyse readily with an equivalent amount of hydrochloric acid, but their inactivity was not surprizing in view of the nature of the mustard moiety, against various tumor test systems.

Certain enamine mustards of a type(LVII) were shown to hydrolyse faster at pH 6 than at pH 7.9 as that of HN2(137).

\[ (\text{ClCH}_2\text{CH}_2)_2\text{NCH} = \text{CH}_2\text{COCH}_3. \]

(LVII)

Complete inhibition of Leukemia L 1210, as well as activity against other murine tumors, has been achieved, with carboxamide, a nitrogen mustard derivative, which is structurally related to the aromatic triazene mustards(138).

These compounds when transferred to water, forms a water soluble ionic compound of undetermined structure(139), the
transformed compounds were inactive in the initial stages. The search for suitable substrates for the phosphoramidases that are reportedly more abundant in neoplastic cells than in normal cells has resulted in the synthesis of numerous phosphorylated nitrogen mustard derivatives.

Cyclophosphamide, cytoxan, endoxan were the potential anticancer compounds, which have been proved to be clinically useful. In a study of changes in a heterocyclic moiety of cyclophosphamide, the O,S-cyclic trimethylene ester of phosphoramidothioic acid exhibited considerable activity against Walker rat carcinoma. The corresponding sulphur analog was just inactive. The derivatives are represented as (LIX).

\[ Z = 0, S, N. \]

Weatherbee et al., reported the compounds in which the principle carrier has been extended with varying results to nitrogen mustards based on hydroquinone, the so called Weatherbee mustard is a unique product.

Steroides as in (LXI) and (LXII) and numerous other
heterocyclic compounds with different structures have been reported.

Interest in nitrogen mustard analogs of heterocyclic materials stemmed from the knowledge that chloroquine and quinacrine localize in cell nuclei. The terminal attachment of the mustard function to the side chains of typical antimalarial drugs resulted in chloroquine (IXIII), quinacrine (IXIV) and camazine (IXV) nitrogen mustards, which inhibit the growth of some leukemias, tumors and showed some evidence of clinical activity (144).

Direct attachment of the mustard group -N(CH\textsubscript{2}CH\textsubscript{2}Cl\textsubscript{2}) to the quinoline ring as in (IXVI), led to inactivity (145).
The compounds presented so far, although differing widely in structural characteristics and other properties, have been similar to each other in that they exhibited cytotoxicity in vitro as well as carcinostatic activity in vivo. Much efforts have been expended on the development of "transport forms" of alkylating agents that would require activation in the body before becoming cytotoxic. It was hoped to design compounds that would be activated selectively by neoplastic cells with the aid of hydrolytic enzymes not present or not as active in normal cells. Many latent or at least theoretically latent alkylating agents have been designed and tested.

GENERAL PRINCIPLES OF CANCER CHEMOTHERAPY

On the basis of the experimental work and the clinical chemotherapy certain general principles have been drawn which may help in elaborating the adequate treatment and to explain the results achieved.

1. The effect of chemotherapy is, as a rule, inversely proportional to the size of the tumor mass: the greater the mass the less the effect, and vice versa. The reason is that the therapeutic effect of chemotherapeutic agents is based on their chemical interaction with the cell
constituents of importance to its initial activities. Thus alkylating agents enter into covalent bonds with DNA, RNA, proteins and lipids. The most important for the therapeutic effect is apparently the alkylation of DNA, which after several stages leads to fragmentation of high polymeric molecule (146).

2. Antimetabolites enter into easily or hardly reversible complexes with active centres of the enzymes, blocking at some stage the reaction catalysed by the enzymes. With a good imitation of the substrate, the antimetabolite may also be incorporated into the products of the reaction (147).

3. The activity of the agents on a molar basis varies greatly and depends either on the chemical reactivity of the alkylating group under physiological conditions or on the degree of affinity of the antimetabolites with the enzyme. But, regardless, of this, the end result corresponds to the percentage of damaged DNA or enzyme molecules (148). Naturally, when the mass of the tumor tissue is large some of the DNA or the enzyme molecules fail to react with the agent, and as a result the amount of damage and percentage of damaged cells are lower and the end effect is less pronounced. On the other hand, the mass of normal tissue involved inside reaction with the agent is fairly constant, so that the total dose of the agent cannot be increased beyond the amount that damages normal tissue cells within
acceptable limits. It follows that the maximum tolerable dose of the agent is a relatively constant value. This shows that:

(a) If the mass of the neoplastic tissue is large no greater effect can be expected from the use of chemotherapy-not-complete regression of the tumors.

(b) To produce the maximum effect, chemotherapy must be limited at earlier stages, or the amount of tumor tissue reduced before hand by non-radical surgical intervention or the treatment administered in several successive courses, thereby systematically diminishing the mass of neoplastic tissue.

(c) And lastly to check the result of chemotherapy, it is necessary to observe the side effects of the drug or the compound administered to the affected body.

Other things which are equally important, the effect of chemotherapy with a given compound depends upon whether it is administered for the primary tumor or for the metastases and on the location of the latter. Primary tumors are less sensitive than metastases to some agents. The effect of chemotherapy on metastases also depends upon the organ in which they are localized. So the effectiveness of the compound should be appraised separately, in relation to primary tumors and to metastases(149).

In addition to this few other principles have been summarised here:

(a) It has been observed that no chemical compound has been found that is capable of curing any form of cancer, although a number of compounds which have come into existence increases the survival time to certain extent;
(b) that neoplastic cells cannot be considered truly "foreign" to the host; and

(c) lastly the biological characteristics of malignant cells are qualitatively indistinguishable from those of the normal cells.

It is obvious that the selective action of an agent on one cell in the presence of other cells must depend ultimately on a difference between the target cells and other cells in at least one biochemical process. This difference may be quantitative and may relate to:

(i) the transport of the agent into the cell;

(ii) activation of deactivation of the agent within the cell;

(iii) a vital regulatory process in the cell, or

(iv) the secondary structure of a macromolecule of the cell.

If well defined difference between a cancer cell and a host normal cell was known, it is almost certain that a compound eventually could be designed that would exploit this difference for the selective inhibition or destruction of the cancer cell.

The difficulty in defining a metabolic basis for cancer chemotherapy is apparent, if one contrasts chemotherapy of cancer with the chemotherapy of infection diseases. In infections, the target of chemotherapy is a foreign, invading organism that is expected to have not one but many metabolic features different from that of host cells. The metabolic change or group of changes that are responsible
for the conversion of normal cell to a cancer cell, the cells of a developed tumor which is the object of chemotherapy, related to the primary biochemical lesion, may be critical for the rate of growth of the tumor and hence of importance to chemotherapy.

An effective chemotherapeutic agent should, therefore, possess highly selective activity to kill the ineffective organism in concentrations which are relatively harmless to the tissue of the host. Another problem of chemotherapy is that of selective toxicity. The parasite, whether exogenous (bacterium : virus) or endogenous in origin (neoplastic cell), has to be destroyed completely, while inflicting only a minimum of damage of the host. The interaction of the administered drug with a receptor, present in the parasite and essentially for its survival but absence from the host, would constitute an ideal situation.

COMBINATION OF CHEMOTHERAPY AND RADIOTHERAPY

The result of different kinds of radiotherapy employed in combination with chemotherapy are encouraging. The first chemotherapeutic agent, nitrogen mustard and its derivatives were tested on patients on whom the resources of radiotherapy had already been exhausted, the disease was generalized, and the hemopoietic system was depressed both by the disease itself and by repeated exposure to ionizing radiations.
In the mean time it was demonstrated that the drug treatment of Hodgkin's disease, if instituted at early stage, without radiation treatment, and with rational technique, produced even better long term results as shown by Larionov and Ziv (1958) (15). The treatment should begin with chemotherapy and radiotherapy should be administered in addition, if necessary, to unresolve nodes or their remains. A large number of reviews and papers have been published with this aspect of the problem (11b).

**COMBINATION OF CHEMOTHERAPY AND SURGERY**

Chemotherapy may be combined with surgery in various ways. It may be used as pre-operatively, for example, to reduce the size and increase the mobility of in-operable tumors or to get rid of ascites, in order to make operation feasible. In this case chemotherapy acts as surgery's helper by increasing the indications for it and making surgical intervention a more promising prospect.

Sometimes, the surgery becomes the helper to chemotherapy, e.g., when the operation removes the latter portion of the tumors, their size sharply diminishes and subsequently chemotherapy becomes more effective. This technique is known as post-operative. However, it cannot as yet be considered definitely established clinically that post-operative chemotherapy produces useful results in preventing
local relapses and late metastases, although there are some observations to that effect (152).

MODE OF ACTION OF ALKYLATED AGENTS

The chemotherapeutic alkylating agents are chemically reactive compounds that combine most readily with nucleophilic centres, a fully saturated carbon atom of the alkylating group becoming attached to the nucleophile. Alkylations of biological interest involve attack at the nitrogen or oxygen atoms of basically important functional groups, such as amino groups, thiolate anions of nucleotides. The studies of Brooks and Lawley (1961, 1964) (88, 89, 72), and Lawley and Brooks (1965) (90) on the interaction of alkylating agents with DNA, suggest that the key biological compounds alkylated in the purine base, guanine in which 7 nitrogen is strongly nucleophilic. The possible consequences of the reaction of nitrogen mustards with guanine residues in DNA chains follow the path as explained in Scheme A. Firstly one 2-chloroethyl side chain undergoes a first order $S_N^1$ intramolecular cyclisation, with release of a chloride ion and formation of highly reactive ethylenimmonium intermediate. By this reaction the tertiary amine is converted to a quaternary ammonium compound. These intermediate compound form carbonium ions, which can react quickly with a large number of organic radials by a second order $S_N^2$ nucleophilic substitution reaction. The
rate controlling step of the $S_{N2}$ alkylation itself involves simultaneous bond forming and bond breaking processes as the nucleophile approaches the alkylating agent, the rate being dependant on the concentrations of both. Normally, guanine residues in DNA exist predominantly in the keto tautomer and readily make Watson-Crick base pairs by hydrogen bonding with cytosine residues. However, when the 7 nitrogen of guanine is alkylated (to become a quaternary ammonium nitrogen), the guanine residue is more acidic and enol tautomer is favoured. Guanine in this form can make base pairs with thymine residues, thus leading to possible miscoding and the ultimate substitution of an adenine-thymine base pair for a guanine-cytosine base pair. Secondly, alkylation of the 7 nitrogen labilizes with imidazole ring, making possible of the opening of the imidazole ring or depurination by excision of guanine residues, either of which can cause serious damage to DNA molecule (153) and thirdly, with bifunctional alkylating agents, such as nitrogen mustards, the second 2-chloroethyl-side chain can also go under similar cyclization and can alkylate another nucleophilic moiety, such as amino groups or a radical of protein. This results into the cross linking of two nucleic acid chains or the linking of a nucleic acid to a protein by a very strong covalent bonds which causes a major disruption in nucleic acid function.
Any of these effects could adequately explain both the mutagenic and cytotoxic effects of alkylating agents, which are the requisite for the cancer activity.

Although bifunctionality is not a pre-requisite for significant anticancer activity, the most active agents are bifunctional agents. With respect to mutagenesis some monofunctional agents are as active as, or more active than, their bifunctional counterparts, but with respect to cytotoxicity the greater effectiveness of bifunctional agents has been attributed to their ability to cross-link twin strands of DNA. Factors such as pH and concentration of reactants affect not only the reaction rates of alkylating agents, and hence sometimes the apparent order of reaction, but also the reactivity of competing nucleophiles. As nucleophiles, thiols, and carboxylic acids are more reactive, when ionized, whereas amines are reactive as the free bases and unreactive in the protonated form. Alkylating agents in general show a reaction preference for sulphur, nitrogen and oxygen, in that order, and under physiological conditions, as formed inside cells, alkylating agents would show the following order of preference for the major nucleophiles, if such were equally accessible and present in equal concentration: ionized thiol > amine > ionized phosphate > ionized carboxylic acid.
Mode of action of alkylating agents

Keto tautomer favoured

Enol tautomer favoured

Cross linking with second guanine

+ Depurinated DNA Chain — DNA Chain Scission.
CHEMICAL REACTIVITY AND CARCINOSTATIC EFFECT

Ross and Colleagues (76) have found that the tendency of alkylating agents, especially the aromatic nitrogen mustards, to become reactive (form carbonium ions) can be expressed by the rate of hydrolysis in aqueous acetone. The reactivity of functional group appears to determine the biological activity of these compounds. Since the reactivity is a function of the basicity of the central N (its electron releasing capacity), substitution at this atom may profoundly influences both chemical and biological activity. Activation can be brought about by introduction of electron-repelling (e.g., methyl, methoxy) groups and deactivation by electron-attracting (e.g., chloro, carbethoxy, aldehydo, or nitro) groups. Apparently a certain minimum chemical reactivity is required to produce the desired biological effect ensues when the reactivity is too high.

The varieties of alkylating functions and the multitude of carrier molecules that characterise these carcinostatic agents, it might be expected that another effective anticancer compound results every time two 2-chloroethyl groups meet on a nitrogen atom, one or two ethylenimine rings are attached to strategically placed carbon atoms or two methane-sulfonate groups find themselves in appropriate proximity to each other. The literature of
experimental cancer chemotherapy is replete with reports on the many hundreds of derivatives that despite structural resemblance to the active compounds, failed to affect the growth of any tumor at tolerated doses. A thorough knowledge of these unsuccessful products of synthetic efforts, and of the physical, chemical or biochemical reasons for their inactivity, is of prime importance to those engaged in the design and preparation of yet another new collection of putative alkylating agents.

Turning to the carcinostatic activity of the alkylating agents, it is pertinent to ask whether this general class differs significantly in antitumor potency from other types of effective agents, whether there are appreciable differences in therapeutic index of or other criteria of usefulness between individual members of this class, and whether these agents are endowed with the ability to inhibit particular experimental neoplasms in the selection way. A number of recent reviewers like Bergel (1964, 1965) (157); Biesela (1962) (158); Elson (1963) (159); Goldin et al. (1966) (160); Haddow (1959, 1973) (161-162); Jones et al. (1960) (163); Karnofsky and Clarkson (1963) (164); Larionov (1965) (165); Rutman (1966) (166); Sartorelli (1965) (167); Skipper (1964) (168); Skipper et al. (1965) (169); Stock (1959) (170); Sugiuira (1961) (171) and Jones (1973) (172) have discussed some of these problems. The various clinically important agents, used in the treatment of various type of tumors are as shown in Appendix I.
<table>
<thead>
<tr>
<th>Type of Agent</th>
<th>Compounds name (Commercial names)</th>
<th>Usual Dose</th>
<th>Disease Description</th>
<th>Ref. No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitrogen Mustards</td>
<td>Mustergen (Mechlor-ethamine, HN2)</td>
<td>0.4 mg./kg. (single iv dose)</td>
<td>Hodgkin's disease, breast, lymphosarcoma, ovary.</td>
<td>15,173</td>
</tr>
<tr>
<td></td>
<td>Cyclophosphamide (cytoxan, endoxan)</td>
<td>2-8 mg./kg. (daily for 6 days, orally or iv)</td>
<td>Acute and chronic lymphocytic leukemias.</td>
<td>15,174</td>
</tr>
<tr>
<td></td>
<td></td>
<td>30-50 mg./kg. (single iv dose)</td>
<td>Hodgkin's disease, multiple, lymphosarcoma.</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>50-300 mg./kg. (daily orally)</td>
<td>Myeloma, ovary, lung, Wilm's tumor, rhabdomyosarcoma.</td>
<td></td>
</tr>
<tr>
<td>Alkylating Agents</td>
<td>Melphalan (Alkeran: Sarcolysin-1)</td>
<td>6 mg. (orally daily for 2-3 weeks); repeated after interruption of one week</td>
<td>Multiple myeloma, acute lymphocytic leukemia, bone reticulosarcoma, ovarian.</td>
<td>175</td>
</tr>
<tr>
<td></td>
<td>Uracil mustard</td>
<td>1-2 mg. (orally daily for 3 weeks); repeated after interruption of one week</td>
<td>Chronic lymphocytic leukemia, Hodgkin's disease, ovary lymphosarcoma, testis.</td>
<td>81,176</td>
</tr>
<tr>
<td></td>
<td>Chlorambucil (Leukeran)</td>
<td>0.1-0.2 mg./kg. (orally daily for 3-5 weeks)</td>
<td>Chronic lymphocytic leukemia, Hodgkin's disease, breast, lymphosarcoma, ovary, testis.</td>
<td>15,177</td>
</tr>
<tr>
<td>Drug Name</td>
<td>Dose (mg)</td>
<td>Duration</td>
<td>Tumor Type</td>
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<td>---------------------------------------</td>
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<tr>
<td>Degranol (1,6-bis(2-chloroethyl)amino-1, 6-dideoxy-D-mannitol)</td>
<td>100 mg (daily for 6 days orally or iv)</td>
<td>Hodgkin's disease, lymphoreticulosarcoma, rhabdomyosarcoma.</td>
<td></td>
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</tr>
<tr>
<td>Dopan (5-bis(2-chloroethyl)amino-6-methyluracil)</td>
<td>0.5-1.0 mg (daily orally)</td>
<td>Hodgkin's disease, carcinoma, lymphoreticulosarcoma.</td>
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<tr>
<td>Chloromethine oxide (Nitromin)</td>
<td>2.0-2.5 mg (daily orally or iv)</td>
<td>Hodgkin's disease, chronic lymphatic leukemia, chronic myeloid leukemia, lung, ovary, lymphoreticulosarcoma.</td>
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</tr>
<tr>
<td>Tryptophan (5-bis(2-chloroethyl)amino-DL-tryptophan)</td>
<td>0.1-0.2 mg/kg (orally daily for 2-4 weeks)</td>
<td>Chronic lymphocytic leukemia, Hodgkin's disease, carcinoma.</td>
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</tr>
<tr>
<td>Phenesterin (Cholesterol ester of p-di(2-chloroethyl)amino-phenyl acetic acid)</td>
<td>0.2-0.4 mg/kg (orally or iv)</td>
<td>Hodgkin's disease.</td>
<td></td>
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</tr>
<tr>
<td>Spirazidine (N,N-di(2-chloroethyl)-N2,N3-dimethylspirotripiperazinium)</td>
<td>0.2-0.5 mg (daily orally)</td>
<td>Lymphoreticulosarcoma, ovary, carcinoma.</td>
<td></td>
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</tr>
<tr>
<td>BCNU (1,3-bis(2-chloroethyl)-1-nitrosourea)</td>
<td>2.0-2.5 mg/kg (intraperitoneal iv)</td>
<td>Leukemia, lung, spleen, heart, liver, kidney, brain tumor.</td>
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</tr>
<tr>
<td>Quinacrine mustard</td>
<td>0.2-4.0 mg/kg (orally or iv)</td>
<td>Melanoma, seminoma, mammary carcinoma.</td>
<td></td>
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</tr>
<tr>
<td>Chloroquine mustard</td>
<td>0.1-0.3 mg/kg (orally or iv daily)</td>
<td>Hodgkin's disease, lymphosarcoma.</td>
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<tr>
<td>Antimetabolites</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
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<td>-----------------------------------------------------</td>
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</tr>
<tr>
<td><strong>Thio-TEPA</strong> (N,N,N&quot;-Triethylenetriphosphoramid)</td>
<td>0.5 mg. (orally administered daily)</td>
<td>Myeloblastic, promyelocytic, myelocytic, lymphatic leukemias, Hodgkin's disease, reticulosarcoma, Brill-Symmer's disease, breast, ovary, uterus, prostate.</td>
<td>184</td>
<td>185</td>
</tr>
<tr>
<td><strong>Aze-TEPA</strong></td>
<td>0.4-0.6 mg. (orally administered)</td>
<td>-do-</td>
<td>186</td>
<td></td>
</tr>
<tr>
<td><strong>TEM(2,4,6-Triethyl-eneimino-sym-triazine)</strong></td>
<td>(orally daily)</td>
<td>Myeloblastic leukemia, ovary, promyelocytic leukemia, myelocytic lymphatic leukemia, polyeythemia, reticulosarcoma, Brill-Symmer's disease, breast.</td>
<td>187</td>
<td></td>
</tr>
<tr>
<td><strong>Trenimon</strong></td>
<td>(intravenous)</td>
<td>-do-</td>
<td>188</td>
<td></td>
</tr>
<tr>
<td><strong>Folic acid analog</strong></td>
<td>20.5-5.0 mg. (orally daily)</td>
<td>Myeloblastic, promyelocytic, myelocytic leukemias.</td>
<td>189</td>
<td></td>
</tr>
<tr>
<td><strong>Thioguanine</strong></td>
<td>25.0-30.0 mg. (iv weekly)</td>
<td>Acute lymphocytic leukemia, choriocarcinoma, mycosis fungoides, breast, testis, oropharyngeal.</td>
<td>174c,190</td>
<td></td>
</tr>
<tr>
<td><strong>Purinethol</strong></td>
<td>50.0-100.0 mg. (orally daily 4 weeks)</td>
<td>Mycosis fungoides, polythemia vera.</td>
<td>191</td>
<td></td>
</tr>
<tr>
<td><strong>Azauridine(Triacetyl-6-azauridine;Triazue)</strong></td>
<td>270.0 mg./kg. (orally daily)</td>
<td>-do-</td>
<td>191</td>
<td></td>
</tr>
<tr>
<td></td>
<td>135.0 mg./kg. (daily orally)</td>
<td>-do-</td>
<td>191</td>
<td></td>
</tr>
<tr>
<td>Drug Class</td>
<td>Drug Name</td>
<td>Dosage</td>
<td>Comments</td>
<td></td>
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<tr>
<td>5-Fluorouracil</td>
<td>1.0-2.0 mg./kg. (iv daily for 4-5 days)</td>
<td>Breast, colon, stomach, ovary, oropharyngeal, urinary bladder.</td>
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<td></td>
<td>(5-Fu; fluorouracil; FUDR)</td>
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<td></td>
<td>Cytarabine (Cytosine arabinoside; ARA-C)</td>
<td>2.0-4.0 mg./kg. (iv daily for 10-20 days)</td>
<td>Acute lymphocytic and acute granulocytic leukemias.</td>
<td></td>
</tr>
<tr>
<td>Purine Analogs</td>
<td>2.5 mg./kg. (orally daily)</td>
<td>Acute lymphocytic and chronic granulocytic leukemias, choriocarcinoma.</td>
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<td></td>
<td>6-Thioguanine (T.G.)</td>
<td></td>
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<tr>
<td>Natural Products</td>
<td>Vinblastine (VLB; VLBAN)</td>
<td>0.1-0.3 mg./kg. (iv weekly)</td>
<td>Hodgkin's disease, breast, lymphosarcoma, choriocarcinoma.</td>
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<td></td>
<td>Vincristine (VCR; ONCONIN)</td>
<td>0.01-0.03 mg./kg. (adults; upto 0.1 mg./kg. iv weekly)</td>
<td>Acute lymphocytic leukemia, neuroblastoma, Wilm's tumor, sarcoma.</td>
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<td></td>
<td>Colchicine (Demecolein; SPG; SANDOZ)</td>
<td>0.1-0.4 mg./kg. (daily)</td>
<td>Skin, esophagus, ovary, prostate, uterus, seminoma.</td>
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<td>5</td>
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<tr>
<td><strong>Antibiotics</strong></td>
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<tr>
<td>Actinomycin D</td>
<td></td>
<td>40.0-60.0 mg./kg. (per day iv)</td>
<td>Wilm's tumor, neuroblastoma, rhodomyosarcoma, botryoides, choriocarcinoma, malignant lymphoma, testis, breast.</td>
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<tr>
<td>(Dactinomycin; COSMOSGEN)</td>
<td></td>
<td>400 mg./kg. (per day orally for 7 days)</td>
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<tr>
<td>Mitomycin C</td>
<td></td>
<td>40.0-50.0 mg. (per day iv)</td>
<td>Rhodomyosarcoma, Hodgkin's disease, granulocytic, multiple myeloma, mammary, bronchogenic carcinoma.</td>
<td></td>
</tr>
<tr>
<td>Streptonigrin</td>
<td></td>
<td>100-150 mg./kg. (per day iv)</td>
<td>Hodgkin's disease, breast, malignant lymphoma, kidney, liver.</td>
<td></td>
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<tr>
<td>Daunomycin</td>
<td></td>
<td>1.0-2.0 mg./kg. (orally daily for 1-2 weeks or iv)</td>
<td>Kidney, liver</td>
<td></td>
</tr>
<tr>
<td>Enzymes</td>
<td></td>
<td>0.015 mg./kg. (iv daily)</td>
<td>Acute lymphocytic leukemia</td>
<td></td>
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<tr>
<td><strong>Hormones</strong></td>
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<tr>
<td>Progestin</td>
<td>Hydroxyprogesterone caproate (DEIALUTIN)</td>
<td>1.0 g. (im twice weekly)</td>
<td>Endometrium</td>
<td></td>
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<tr>
<td>Medroprogestosterone acetate</td>
<td></td>
<td>100-200 mg. (daily or twice weekly im)</td>
<td>Chronic lymphocytic leukemia</td>
<td></td>
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<tr>
<td>Cortisol</td>
<td></td>
<td>25-37.5 mg./kg. (7 doses orally)</td>
<td>-do-</td>
<td></td>
</tr>
<tr>
<td>Prednisolone (B-Cortef)</td>
<td></td>
<td>50-100 mg. (orally per day)</td>
<td>lymphosarcoma, leukemia</td>
<td></td>
</tr>
<tr>
<td>Treatment</td>
<td>Drug/Drug Formulation</td>
<td>Dosage/Route</td>
<td>Indications</td>
<td></td>
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<tr>
<td>Adrenocorticosteroids</td>
<td>Prednisone (METICORTEN)</td>
<td>20-100 mg. (orally daily or every third day)</td>
<td>Acute and chronic lymphocytic leukemia, chronic granulocytic leukemia, lymphosarcoma, breast.</td>
<td></td>
</tr>
<tr>
<td>Estrogens</td>
<td>Diethylstilbestrol</td>
<td>1.5 mg. (orally 3 times daily)</td>
<td>Breast, prostate</td>
<td></td>
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<tr>
<td></td>
<td>Ethinylestradiol (ESTINYL)</td>
<td>1.0 mg. (orally 3 times daily)</td>
<td>Carcinoma of cervix</td>
<td></td>
</tr>
<tr>
<td>Androgens</td>
<td>Testosterone propionate</td>
<td>50-100 mg. (im 3 times weekly)</td>
<td>Breast</td>
<td></td>
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<tr>
<td></td>
<td>Fluoxymesterone (HALOTESTIN)</td>
<td>10-20 mg. (orally daily)</td>
<td>Advanced breast cancer</td>
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<tr>
<td></td>
<td>17-Methyl testosterone</td>
<td>200 mg. per day</td>
<td>Advanced breast cancer</td>
<td></td>
</tr>
<tr>
<td>ACTH</td>
<td>ACTH (PORCINE)</td>
<td>50-100 mg. (orally 7 doses)</td>
<td>Acute leukemia, breast</td>
<td></td>
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<tr>
<td>Miscellaneous</td>
<td></td>
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<tr>
<td>Substituted urea</td>
<td>Hydroxy urea (HYDREA)</td>
<td>20-80 mg./Kg. (orally every 3 day)</td>
<td>Chronic granulocytic leukemia, malignant melanoma.</td>
<td></td>
</tr>
<tr>
<td>Methyl hydrazine derivative</td>
<td>Procarbazine (NATULAN)</td>
<td>50-300 mg. (orally daily)</td>
<td>Hodgkin's disease</td>
<td></td>
</tr>
<tr>
<td>Radioactive isotopes</td>
<td>Sodium phosphate $^{32}$P</td>
<td>2.5-5.0 mc (iv single dose)</td>
<td>Polyesthemia vera, chronic lymphatic and granulocytic leukemias.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sodium iodide $^{131}$I</td>
<td>100-200 mc (orally or iv)</td>
<td>Thyroid</td>
<td></td>
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</tbody>
</table>