GOODMAN and GILMAN's
The Pharmacological Basis of Therapeutics

SEVENTH EDITION

MACMILLAN PUBLISHING COMPANY
New York

COLLIER MACMILLAN CANADA, INC.
Toronto

COLLIER MACMILLAN PUBLISHERS
London

MACMILLAN PUBLISHING COMPANY
866 Third Avenue • New York, N.Y. 10022

COLLIER MACMILLAN CANADA, INC.

COLLIER MACMILLAN PUBLISHERS • London

Library of Congress catalog card number 85-15356

Printing: 1 2 3 4 5 6 7 8 Year: 5 6 7 8 9 0 1

In this textbook, reference to proprietary names of drugs is ordinarily made only in chapter sections dealing with preparations. Such names are given in SMALL-CAP TYPE, usually immediately following the official or nonproprietary titles. Proprietary names of drugs also appear in the Index.

Drugs currently used in chemotherapy of neoplastic diseases may be divided into several classes, as shown in Table XIII–1. This somewhat arbitrary classification is used in Chapter 55 as a convenient framework for describing the various types of agents; the major clinical indications for the drugs are listed in Table XIII–1 in order to facilitate rapid reference. Dosage regimens, which are often complex, are discussed under the individual drugs.

Mechanistic classification of these agents is increasingly important, particularly as investigators attempt to utilize this information to design "rational" regimens for chemotherapy. A simplified overview of the sites of action of many of the drugs described in Chapter 55 is shown in Figure XIII–1.

CHAPTER 55 ANTIPROLIFERATIVE AGENTS AND DRUGS USED FOR IMMUNOSUPPRESSION

Paul Calabresi and Robert E. Parks, Jr.

I. Alkylating Agents

History. Although synthesized in 1854, the vesicant properties of *sulfur mustard* were not described until 1887. During World War I, medical attention was first focused on the vesicant action of sulfur mustard on the skin, eyes, and respiratory tract. It was appreciated later, however, that serious systemic intoxication also follows exposure. In 1919, Krumbhaar and Krumbhaar made the pertinent observation that the poisoning caused by sulfur mustard is characterized by leukopenia and, in cases that came to autopsy, by aplasia of the bone marrow, dissolution of lymphoid tissue, and ulceration of the gastrointestinal tract.

In the interval between World Wars I and II, extensive studies of the biological and chemical actions of the *nitrogen mustards* were conducted. The marked cytotoxic action on lymphoid tissue prompted Gilman, Goodman, and T. F. Dougherty to study the effect of nitrogen mustards on transplanted lymphosarcoma in mice, and in 1942 clinical studies were initiated. This launched the era of modern cancer chemotherapy (Gilman, 1963).

In their early phases, all these investigations were conducted under secrecy restrictions imposed by the use of classified chemical-warfare agents. At the termination of World War II, however, the nitrogen mustards were declassified and a general review was presented by Gilman and Philips (1946), and shortly thereafter there appeared summaries of clinical research by Goodman and associates (1946), Jacobson and coworkers (1946), and Rhoads (1946). Recent reviews include those by Colvin (1982), Wheeler (1982), Connors (1983), and Ludlum and Tong (1985).

Thousands of variants of the basic chemical structure of the nitrogen mustards have been prepared. However, most attempts at the rational design of "active-site-directed" molecules have failed, and only a few of these agents have proven more useful than the original compound in specific clinical circumstances (*see* below). At the present time five major types of alkylating agents are used in the chemotherapy of neoplastic diseases: (1) the nitrogen mustards, (2) the ethylenimines, (3) the alkyl sulfonates, (4) the nitrosoureas, and (5) the triazenes.

Chemistry. The chemotherapeutic alkylating agents have in common the property of undergoing strongly electrophilic chemical reactions through the formation of carbonium ion intermediates or of transition complexes with the target molecules. These reactions result in the formation of covalent linkages (alkylation) with various nucleophilic substances, including such biologically important moieties as phosphate, amino, sulfhydryl, hydroxyl, carboxyl, and imidazole groups. The cytotoxic and other effects of the alkylating agents are directly related to the alkylation of components of DNA. The 7 nitrogen atom of guanine is particularly susceptible to the formation of a covalent bond with both monofunctional and bifunctional alkylators and may well represent the key target that determines the biological effects of these agents. It must be appreciated, however, that other atoms in the purine and pyrimidine bases of DNA—for example, the 1 or 3 nitrogens of adenine, the 3 nitrogen of cytosine, and the 6 oxygen of guanine—may also be alkylated to a lesser degree, as are the phosphate atoms of the DNA chains and the proteins associated with DNA.

To illustrate the actions of alkylating agents, possible consequences of the reaction of mechlorethamine (nitrogen mustard) with guanine residues in DNA chains are shown in Figure 55–1. First, one 2-chloroethyl side chain undergoes a first-order (S_N1) intramolecular cyclization, with release of a chloride ion and formation of a highly reactive ethylenimonium intermediate. By this reaction the tertiary amine is converted to a quaternary ammonium compound. The ethylenimonium intermediates can react avidly, through formation of a carbonium ion or transition complex intermediate, with a large number of inorganic ions and organic radicals by reactions that resemble a second-order (S_N2) nucleophilic substitution reaction (Price, 1975). Alkylation of the 7 nitrogen of guanine residues in DNA, a highly favored reaction, may exert several effects of considerable biological importance, as illustrated in Figure 55–1. Normally, guanine residues in DNA exist predominantly as the keto tautomers 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 the enol tautomer is favored. 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. Second, alkylation of the 7 nitrogen labilizes the imidazole ring, making possible the opening of the imidazole ring or depurination by excision of guanine residues, either of which can result in serious damage to the DNA molecule

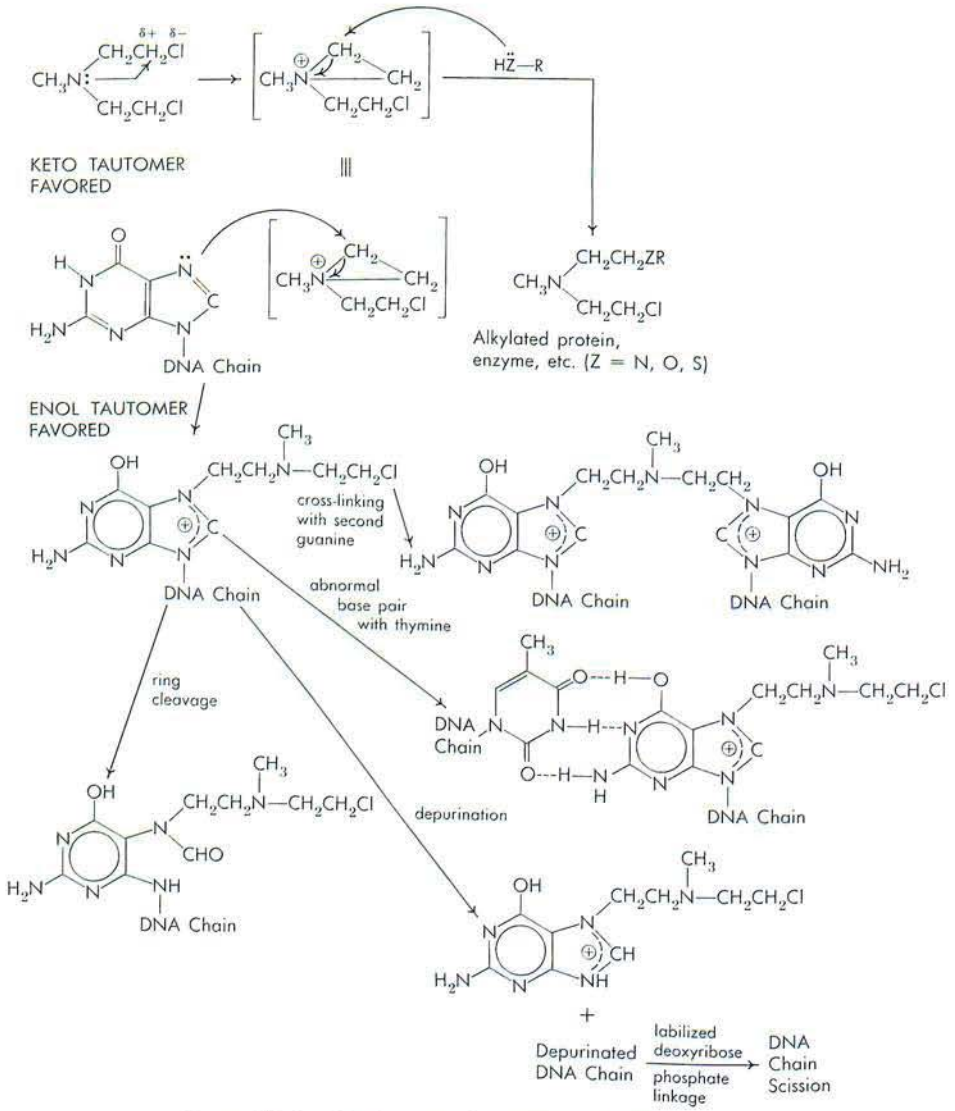


Figure 55–1. *Mechanism of action of alkylating agents.*

(Shapiro, 1968). Third, with bifunctional alkylators, such as nitrogen mustard, the second 2-chloroethyl side chain can undergo a similar cyclization reaction and alkylate a second guanine residue or another nucleophilic moiety, such as an amino group or a sulfhydryl radical of a protein. This can result in the cross-linking of two nucleic acid chains or the linking of a nucleic acid to a protein by very strong covalent bonds, reactions that would cause a major disruption in nucleic acid function. Any of these effects could adequately explain both the mutagenic and the cytotoxic effects of alkylating agents.

In addition to the formation of covalent bonds with purine or pyrimidine residues of DNA, a wide variety of other chemical reactions are possible that can result in a number of other important effects on cellular function and viability.

All nitrogen mustards are chemically unstable but vary greatly in their degree of instability. Therefore, the specific chemical properties of each member of this class of drugs must be considered individually in therapeutic applications. For example, *mechlorethamine* is so hygroscopic and unstable in aqueous form that it is marketed as the dry crystals of the hydrochloride salt. Solutions are prepared immediately prior to injection and, within a few minutes after administration, mechlorethamine reacts almost completely within the body. On the other hand, agents such as *chlorambucil* are sufficiently stable to permit oral administration, and *cyclophosphamide*, which is much less reactive than mechlorethamine, requires biochemical activation by the cytochrome P-450 system of the liver in order to achieve chemotherapeutic effectiveness.

The ethylenimine derivatives react by an S_N2 reaction; however, since the opening of the ethylenimine ring is acid catalyzed, they are more reactive at acidic pH. Busulfan is an atypical alkylating agent with unusual biological properties that differ significantly from substituted nitrogen mustards and ethylenimines (Fox, 1975).

Structure-Activity Relationship. The alkylating agents used in chemotherapy encompass a diverse group of chemicals that have in common the capacity to contribute, under physiological conditions, alkyl groups to biologically vital macromolecules such as DNA. In most instances, physical and chemical parameters, such as lipophilicity, capacity to cross biological membranes, acid dissociation constants, stability in aqueous solution, and so forth, rather than similarity to cellular constituents, have proven crucial to biological activity. With several of the most valuable agents, for example, cyclophosphamide and the nitrosoureas, the active alkylating moieties are generated *in vivo* following complex degradative reactions, some of which are enzymatic. Since many of these physicochemical factors and activation reactions are still unclear, most alkylating agents in use today were discovered by empirical rather than by rational approaches. In most instances where clinically useful agents were uncovered by presumably "rational" methods, it was later learned that the original premises were defective, and the biological usefulness resulted from factors not considered in the original design.

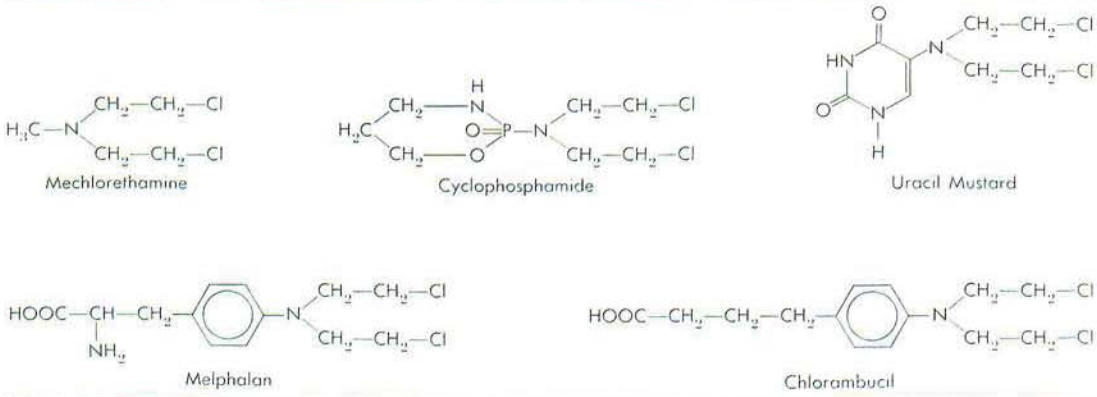
The nitrogen mustards may be regarded as nitrogen analogs of sulfur mustard. The biological activity of both types of compounds is based upon the presence of the *bis*-(2-chloroethyl) grouping. In sulfur mustard, the two reactive groups are attached to bivalent sulfur; since nitrogen is trivalent, a third substituent must be present on the nitrogen atom. Although a very large number of alkylating agents have been synthesized and evaluated, the methyl derivative, *mechlorethamine*, has received wide clinical use and has been accepted generally as a standard of reference. Various structural modifications have been made in order to achieve greater selectivity and, therefore, less toxicity. *Bis*-(2-chloroethyl) groups have been linked to (1) amino acids (phenylalanine, glycine, DL-alanine); (2) substituted phenyl groups (aminophenyl butyric acid, as in *chlorambucil*); (3) pyrimidine bases (uracil); (4) benzimidazole; (5) antimalarial agents; (6) sugars (mannitol); and (7) several other substances, including a cyclic phosphamide ester. Although none of these modifications has achieved the goal of producing a highly selective and general cytotoxic action for malignant cells, some of the compounds exhibit notable differences in their secondary pharmacological properties and have attracted much clinical, as well as theoretical, interest.

The structural formulas of some of the more commonly used nitrogen mustards are shown in Table 55–1.

There is no definite evidence that the use of special prosthetic groups, such as phenylalanine, a precursor of melanin, conveys unusual selectivity of action on malignant melanoma. The addition of substituted phenyl groups has produced a series of derivatives that retain the ability to react by an S_N1 mechanism; however, the electron-withdrawing capacity of the aromatic ring greatly reduces the rate of carbonium ion formation, and these compounds can therefore reach distant sites in the body before reacting with components of blood and other tissues. Chlorambucil is the most successful example of such aromatic mustards. These molecular modifications of mechlorethamine have not altered its general spectrum of action; however, by reducing the high reactivity characteristic of the parent compound, the derivatives may be administered orally and are more convenient in the treatment of chronic malignancies of the lymphocytic or plasma-cell series, particularly in the presence of extensive infiltration of the bone marrow.

A classical example of the role of the host metabolism in the activation of an alkylating agent is seen with *cyclophosphamide*—now the most widely used agent of this class. The original rationale that guided design of this molecule was twofold. First, if a cyclic phosphamide group replaced the N-methyl of mechlorethamine, the compound might be relatively inert, presumably because the *bis*-(2-chloroethyl) group of the molecule could not ionize until the cyclic phosphamide was cleaved at the phosphorus-nitrogen linkage. Second, it was hoped that neoplastic tissues might possess high phosphatase or phosphamidase activity capable of accomplish-

Table 55–1. NITROGEN MUSTARDS EMPLOYED IN THERAPY



ing this cleavage, thus resulting in the selective production of an activated nitrogen mustard in the malignant cells. In accord with these predictions, cyclophosphamide displays only weak cytotoxic, mutagenic, or alkylating activity and is relatively stable in aqueous solution. However, when administered to experimental animals or patients bearing susceptible tumors, marked chemotherapeutic effects, as well as mutagenicity and carcinogenicity, are seen. Although a definite role for phosphatases or phosphamidases in the mechanism of action of cyclophosphamide has not yet been demonstrated, it is clearly established that the drug initially undergoes metabolic activation by the cytochrome P-450 mixed-function oxidase system of the liver, with subsequent transport of the activated intermediate to sites of action, as discussed below. Thus, a crucial factor in the structure-activity relationship of cyclophosphamide concerns its capacity to undergo metabolic activation in the liver, rather than to alkylate malignant cells directly. It also appears that the selectivity of cyclophosphamide against certain malignant tissues may result in part from the capacity of normal tissues, such as liver, to protect themselves against cytotoxicity by further degrading the activated intermediates.

Although initially considered as an antimetabolite, the triazene derivative 5-(3,3-dimethyl-1-triazeno)-imidazole-4-carboxamide, usually referred to as *dacarbazine* or DTIC, is now known to function through alkylation. Its structural formula is as follows:

Dacarbazine

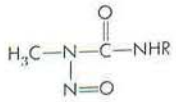
This compound bears a striking resemblance to the known metabolite 5-aminoimidazole-4-carboxamide (AIC), which is capable of conversion to inosinic acid by enzymes of purine synthesis. Thus, it was suspected that dacarbazine acts by inhibiting purine metabolism and nucleic acid synthesis. This resemblance to AIC may be fortuitous, since, for chemotherapeutic effectiveness, dacarbazine requires initial activation by the cytochrome P-450 system of the liver through an N-demethylation reaction. In the target cell, there then occurs a spontaneous cleavage liberating AIC and an alkylating moiety, presumably diazomethane (Chabner, 1982d; Oliverio, 1982).

Although the mechanism of action is not yet fully established, it is generally assumed that the *nitrosoureas*, which include compounds such as 1,3-*bis*-(2-chloroethyl)-1-nitrosourea (carmustine, BCNU), 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea (lomustine, CCNU), and its methyl derivative (semustine, methyl-CCNU), as well as the antibiotic *streptozocin* (*streptozotocin*), exert their cytotoxicity through the liberation of alkylating and carbamoylating moieties. Their structural formulas are shown in Table 55–2.

The antineoplastic nitrosoureas have in common the capacity to undergo spontaneous, nonenzymatic degradation with the formation of a variety of products. Of these, the methyl carbonium ion (from MNU compounds) and the 2-chloroethyl carbonium ion (from CNU compounds) are strongly electrophilic and can alkylate a variety of substances, including the purine and pyrimidine bases of DNA. Guanine, cytidine, and adenine adducts have been identified; a number of these are derived from the attachment of the haloethyl group to nucleophilic sites on purines or pyrimidines in DNA. Displacement of the halogen atom can then lead to interstrand or intrastrand cross-linking of the DNA. The formation of the cross-links after the initial alkylation reaction is a relatively slow process and can be interrupted by a DNA repair enzyme. As with the nitrogen mustards, it is generally agreed that interstrand cross-linking is associated with the cytotoxicity of nitrosoureas (Colvin, 1982; Hemminki and Ludlum, 1984).

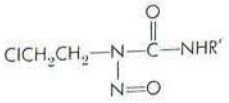
Table 55–2. CLASSIFICATION AND STRUCTURES OF SOME ANTINEOPLASTIC NITROSOUREAS

METHYLNITROSOUREAS (MNU)



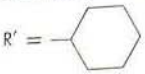
Streptozocin
R = 2-substituted glucose

2-CHLOROETHYLNITROSOUREAS (CNU)



Carmustine (BCNU)
R′ = $-CH_2CH_2Cl$

Lomustine (CCNU)



Semustine (Methyl-CCNU)

Chlorozotocin
R′ = 2-substituted glucose

In addition to the generation of carbonium ions, the spontaneous degradation of nitrosoureas liberates organic isocyanates that are capable of carbamoylating lysine residues of proteins. This reaction can apparently inactivate certain of the DNA repair enzymes, and it has been suggested that high carbamoylating activity might be related to myelosuppression. This has, however, been questioned (*see* Heal *et al.*, 1979). The reactions of the nitrosoureas with macromolecules are shown in Figure 55–2. (For recent reviews of the nitrosoureas, *see* Colvin, 1982; Connors, 1983; Hemminki and Ludlum, 1984; Ludlum and Tong, 1985.)

Since the formation of the ethylenimonium ion constitutes the initial reaction of the nitrogen mustards, it is not surprising that other ethylenimine derivatives or compounds that can produce related structures have antitumor activity. Several agents of this type have been discussed in *earlier editions* of this textbook; these include triethylenemelamine (TEM), triethylenephosphoramide (TEPA), triethylenethiophosphoramide (thiotepa), hexamethylmelamine (HMM), and pentamethylmelamine (PMM). While TEM, TEPA, and thiotepa are cytotoxic, they have no particular clinical advantage over the other alkylating agents. Although there is no evidence that the methylmelamines function as alkylating agents, HMM and PMM are mentioned here because of their chemical similarity to the ethylenimine melamines. The methylmelamines are N-demethylated by hepatic microsomes, with the release of formaldehyde, and there is a relationship between the degree of the demethylation and their activity against murine tumors. HMM requires microsomal activation to display cytotoxicity. The drug appears to have activity against a number of neoplasms that are resistant to

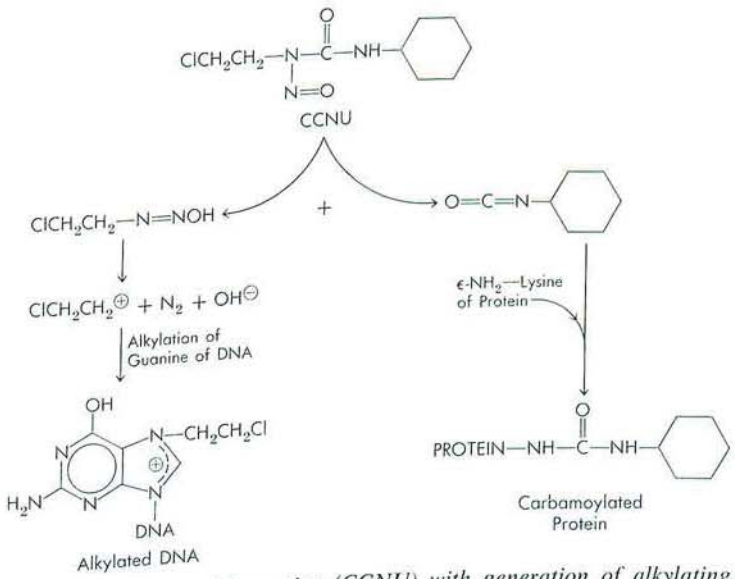
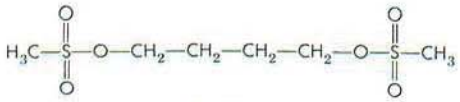


Figure 55–2. *Degradation of lomustine (CCNU) with generation of alkylating and carbamoylating intermediates.*

other alkylating agents. Among these are carcinomas of the ovary, breast, and lung (small cell) and certain lymphomas. Studies are underway to establish the nature of the active metabolite and, hopefully, to identify related compounds with greater therapeutic effectiveness (Rutty and Connors, 1977; Chabner, 1982d).

From a large group of esters of alkanesulfonic acids, synthesized as alkylating agents for chemotherapy of neoplastic disease, several interesting compounds have emerged; one of these, busulfan, is of great value in the treatment of chronic granulocytic leukemia; its structural formula is as follows:



Busulfan

Busulfan is a member of a series of symmetrical methanesulfonic acid esters that permit determination of the effects of altering the length of a bridge of methylene groups (n = 2 to 10); the compounds of intermediate length (n = 4 or 5) possess the highest activities and therapeutic indices. Cross-linked guanine residues have been identified in DNA incubated *in vitro* with busulfan (Tong and Ludlum, 1980).

PHARMACOLOGICAL ACTIONS

The pharmacological actions of the various groups of alkylating agents are considered together in the following discussion. Although there are many similarities, there are, of course, some notable differences. Primary consideration will be given to the cytotoxic actions that follow the administration of a sublethal dose.

Cytotoxic Actions. The most important pharmacological actions of the alkylating agents are those that disturb the fundamental mechanisms concerned with cell growth, mitotic activity, differentiation, and function. The capacity of these drugs to interfere with normal mitosis and cell division in all rapidly proliferating tissues provides the basis for their therapeutic applications and for many of their toxic properties. Whereas certain alkylating agents may have damaging effects on tissues with normally low mitotic indices, for example, liver, kidney, and mature lymphocytes, they are most cytotoxic to rapidly proliferating tissues in which a large proportion of the cells are in division. These compounds may readily alkylate nondividing cells, but cytotoxicity is seen only if such cells are stimulated to divide. Thus, the process of alkylation itself may be a relatively nontoxic event, as long as the DNA repair enzymes can correct the lesions in DNA prior to the next cellular division.

In contrast to many other antineoplastic agents, the effects of the alkylating drugs, although dependent on proliferation, are not cell-cycle specific, and the drugs may act on cells at any stage of the cycle. However, the toxicity is usually expressed when the cell enters the S phase and progression through the cycle is blocked at the G_2 (premitotic) phase (*see* Wheeler, 1967). While not strictly cell-cycle specific, quantitative differences may be detected when nitrogen mustards are applied to synchronized cells at different phases of the cycle. Cells appear more sensitive in late G_1 or S than in G_2, mitosis, or early G_1. Polynucleotides are more susceptible to alkylation in the unpaired state than in the helical form. During replication of DNA, portions of the molecule are so unpaired.

The cells accumulating behind the block at G_2 may have a double complement of DNA while continuing to synthesize other cellular components, such as protein and RNA. This can result in unbalanced growth, with the formation of enlarged or giant cells that can continue to synthesize DNA, making as much as four or five times the normal complement. Lethal cytotoxic action may occur by so-called interphase death and mitotic death; on the other hand, relatively undifferentiated cells of mammalian germinal tissues may remain nonproliferative during exposure and later undergo nuclear and cytoplasmic hypertrophy, differentiating without further mitosis into more adult cell types. Interphase death is generally regarded as the result of damage to many cellular sites. Nevertheless, this may not be the case; certainly it occurs without any evidence of mitotic activity. For detailed reviews of the cytotoxic and biochemical effects of alkylating agents, *see* Connors (1975) and Colvin, (1982).

Biochemical Actions. The great preponderance of evidence indicates that the primary target of pharmacological doses of alkylating agents is the DNA molecule, as illustrated in Figure 55–1. A crucial distinction that must be emphasized is between the bifunctional agents, in which cytotoxic effects predominate, and the monofunctional agents, which have much greater capacity for mutagenesis and carcinogenesis. This suggests that biochemical events such as the cross-linking of DNA strands, only possible with bifunctional agents, represent a much greater threat to cellular survival than do other effects, such as depurination and chain scission. On the other hand, the latter reactions may cause permanent modifications in DNA structure that are compatible with continued life of the cell and transmissible to subsequent generations; such modifications may result in mutagenesis or carcinogenesis (Colvin, 1982; Ludlum and Tong, 1985).

The remarkable DNA repair systems found in most cells appear to play a key, if not determining, role in the relative resistance of nonproliferating tissues, the selectivity of action against particular cell types, and acquired resistance to alkylating agents. While alkylation of a single strand of DNA may often be repaired with relative ease, interstrand cross-linkages, such as those produced by the bifunctional alkylating agents, are more difficult to repair and involve more complex mechanisms. Many of the cross-links formed in DNA by these agents at low doses may also be corrected; higher doses cause extensive cross-linkage, and DNA breakdown occurs.

Detailed information is lacking on mechanisms of cellular uptake of alkylating agents. Mechlorethamine appears to enter murine tumor cells by means of an active transport system, the natural substrate of which is choline. Melphalan, an analog of phenylalanine, is taken up by at least two active transport systems that normally react with leucine and other neutral amino acids. The highly lipophilic nitrosoureas, carmustine and lomustine, diffuse into cells passively (Colvin, 1982).

Mechanisms of Resistance to Alkylating Agents. Acquired resistance to alkylating agents is a common event, and the acquisition of resistance to one alkylating agent may impart cross-resistance to other alkylators. While definitive information on the biochemical mechanisms of resistance is lacking, several biochemical mechanisms have been implicated in the development of such resistance by tumor cells. In contrast to the development of resistance to antimetabolites, where single-step mutations can result in almost complete resistance to drug effects, the acquisition of resistance to alkylating agents is usually a slower process, not resulting from single biochemical changes. Resistance of this type may represent the summation of a series of changes, none of which by itself can confer significant resistance. Among the biochemical changes identified in cells resistant to alkylating agents are decreased permeation of the drugs and increased production of nucleophilic substances that can compete with the target DNA for alkylation. The administration of cysteine can considerably reduce the antitumor effects of alkylating agents, and there are several examples of animal tumors with acquired resistance that have greater concentrations of free thiol groups than do the sensitive tumor lines from which they were derived. There has been much speculation about the possibility that increased activity of the DNA repair system may permit cells to acquire resistance to alkylating agents. It has been suggested that cellular resistance to cyclophosphamide may result from increased rates of metabolism of the activated forms of the drug to the inactive keto and carboxy metabolites (*see* Figure 55–3, page 1256). In addition, pleiotropic drug resistance has been documented in experimental and human tumor cell lines; such cells have become resistant to several agents with different chemical structures and mechanisms of action (Connors, 1974; Colvin, 1982; Symposium, 1983a).

Hematological and Immunosuppressive Actions. The hematopoietic system is very susceptible to the effects of alkylating agents. Within 8 hours after administration of a sublethal dose of a nitrogen mustard, cessation of mitosis and disintegration of formed elements may be evident in the marrow and lymphoid tissues. Lymphocytes are more sensitive to the destructive action of the mustards and relatively resistant to the effects of busulfan, an action that is considered responsible for the immunosuppressive effects observed with the former group, particularly cyclophosphamide. Busulfan is more toxic to granulocytes, and suitable combinations of busulfan and chlorambucil, an aromatic mustard, can simulate closely the hematological effects of whole-body x-radiation. The effects of chlorambucil are followed by rapid recovery, except in lymphoid organs, whereas depression of hematopoiesis after busulfan occurs more gradually. In patients treated with mechlorethamine, lymphocytopenia is apparent within 24 hours and becomes more severe for 6 to 8 days; within a few days, granulocytopenia is evident and lasts for 10 days to 3 weeks. Variable depression of platelet and erythrocyte counts may occur during the second or third week after therapy; with ensuing regeneration, hematological recovery is complete at the end of 4 to 6 weeks and rebound hyperplasia may be present from the fifth to the seventh week.

Actions on Reproductive Tissues. In women, amenorrhea of several months' duration sometimes follows a course of therapy with alkylating agents. Impairment of spermatogenesis may be noted in men. Interesting differences and similarities have been found between the effects of these agents and x-rays on the stages of spermatogenesis in rodents. Busulfan mimics most closely the effects of radiation by acting on an early stage of spermatogenesis; this results, after 8 weeks, in a systematic sequential depletion of spermatogonia, spermatocytes, spermatids, and spermatozoa. Triethylenemelamine and the aliphatic mustards affect later stages and produce infertility within 4 weeks. On the other hand, cytotoxic doses of phenylalanine mustard and chlorambucil do not interfere with the fertility of male rats.

Actions on Epithelial Tissues. The intestinal mucosa can be damaged by the parenteral administration of minimal lethal doses

of a nitrogen mustard in experimental animals; mitotic arrest, cellular hypertrophy, pyknosis, disintegration, and desquamation of the epithelium are evident. Damage to the hair follicles is much more pronounced with cyclophosphamide than with other mustards and frequently results in alopecia; this effect is usually reversible, even with continued therapy.

Sulfur mustard and the nitrogen mustards are powerful local vesicants. Either direct contact with the compounds or exposure to vapors can lead to serious local reactions. The susceptible tissues are skin, eyes, and respiratory tract. The mustards are not escharotic *per se;* rather, the onset of action is delayed for many hours and the mechanism of tissue injury presumably involves the reaction of their transformation products with essential components of the cell. The vesicant properties of the nitrogen mustards are of concern to the clinician in that local reactions can occur if certain precautions are not observed during the course of administration (*see* below).

Actions on the Nervous System. All nitrogen mustards have effects on the central nervous system (CNS). Nausea and vomiting are prominent side effects, particularly of mechlorethamine, and are presumably the result of CNS stimulation. Convulsions, progressive muscular paralysis, and various cholinomimetic effects have been observed. These effects and a poorly understood "delayed-death" syndrome reported in animals indicate that the cytotoxicity of the alkylating agents extends to cellular functions unrelated to proliferative activity. More detailed descriptions and references appear in the *fourth* and *earlier editions* of this textbook.

NITROGEN MUSTARDS

The chemistry and the pharmacological actions of the alkylating agents as a group, and of the nitrogen mustards, have been presented above. Only the unique pharmacological characteristics of the individual agents are considered below.

MECHLORETHAMINE

Mechlorethamine was the first of the nitrogen mustards to be introduced into clinical medicine and is the most rapidly acting of the drugs in this class. The chemical structure of mechlorethamine has been presented above (*see* Table 55–1).

Absorption and Fate. Severe local reactions of exposed tissues necessitate intravenous injection of mechlorethamine for clinical use. In either water or body fluids, at rates affected markedly by pH, mechlorethamine rapidly undergoes chemical transformation and combines with either water or reactive compounds of cells, so that the drug is no longer present in active form after a few minutes. Indeed, it is possible to protect a given tissue from the effects of the agent by the simple expedient of interrupting the blood supply to the area for a few minutes during and immediately after injection of the drug. Conversely, it is possible, but not always feasible, to localize the action of mechlorethamine or related agents to a large extent in a given tissue by injecting the drug into the arterial blood stream supplying that tissue.

Preparation, Dosage, and Routes of Administration. *Mechlorethamine hydrochloride* (MUSTARGEN) is supplied in vials containing 10 mg of mechlorethamine hydrochloride triturated with 100 mg of sodium chloride. The solution for injection must be freshly prepared before each administration by adding 10 ml of sterile water to the contents of the vial by means of a syringe and needle, with the use of surgical gloves for protection of the hands. The solution should be injected into the tubing of a rapidly flowing intravenous infusion; this not only reduces the possibility of extravasation of the drug but also lowers the concentration of vesicant that comes in contact with the intima of the vein. The exact rate of injection is relatively unimportant, provided it is completed within a few minutes. In patients who have elevated venous pressure in the antebrachial veins because of compression of the great veins by mediastinal tumors, it is advisable to administer the drug through an indwelling catheter inserted into the femoral vein.

A course of therapy with mechlorethamine consists in the injection of a total dose of 0.4 mg/kg of body weight or 10 mg/sq m. Although this total dose may be given in either two or four daily consecutive injections, a single administration is preferable; the therapeutic response is equal, and the patient is spared an additional 2 or 3 days of anorexia, nausea, and vomiting. The recommended total dosage to be given during a single course should be exceeded only by those who are completely familiar with the use of the drug. In the presence of extensive infiltration of bone marrow by neoplastic cells, as is often the case in diffuse lymphoma, it is wise to reduce the dose to 0.3 or even 0.2 mg/kg, at least for the first course of therapy.

A course of mechlorethamine may be repeated only after bone-marrow function has recovered. This is best ascertained by study of the peripheral blood or by evaluation of bone-marrow granulocyte

reserve. Usually, at least 6 weeks should elapse between courses of this agent.

Direct intracavitary administration of the drug (0.2 to 0.4 mg/kg) for malignant effusions, particularly of pleural origin, provides valuable palliation.

Therapeutic Uses and Clinical Toxicity. The beneficial results of mechlorethamine in *Hodgkin's disease* and, less predictably, in other *lymphomas* have been extensively confirmed. Although the drug has been effective alone, current practice favors its use in combination with other agents. In generalized Hodgkin's disease (stages III and IV), the so-called MOPP regimen (the combination of mechlorethamine, vincristine [ONCOVIN], procarbazine, and prednisone) is considered the treatment of choice (DeVita *et al.*, 1972).

In patients with generalized *mycosis fungoides*, very dilute solutions (0.02%) of mechlorethamine may be painted on the involved cutaneous areas with marked beneficial results.

In the treatment of leukemias and related myeloproliferative disorders, mechlorethamine has been superseded by other agents. Although palliative results have been observed in carcinomas of the bronchus, ovary, breast, and other solid tumors, alkylating agents of intermediate or slower reactivity are preferable. (*See* Lane, 1977; Colvin, 1982; Calabresi *et al.*, 1985.)

The major toxic manifestations of mechlorethamine include nausea and vomiting, as well as myelosuppression. Leukopenia and thrombocytopenia constitute the major limitation on the amount of drug that can be given in a single course. Rarely, hemorrhagic complications of nitrogen mustard therapy may be due to hyperheparinemia; in such a circumstance, specific therapy with protamine corrects the hemorrhagic diathesis (Chapter 58).

On rare occasions, a maculopapular *skin eruption* may follow therapy with mechlorethamine. The reaction apparently is not one of the hypersensitivity type, does not necessarily recur with subsequent administration of the drug, and does not provide a contraindication to further therapy. *Herpes zoster* is another type of skin lesion frequently associated with nitrogen mustard therapy. A latent viral infection is not uncommonly present in patients with malignant lymphoma, and therapy with either a nitrogen mustard or radiation may be followed by overt manifestations of the viral disease.

Women should be warned that *menstrual irregularities* may be produced by mechlorethamine, and, since *fetal abnormalities* have been induced in experimental animals, the drug should not be used if pregnancy exists or is suspected. Breast feeding should be terminated before therapy with mechlorethamine is initiated. After a course of therapy, catamenia may be delayed or several consecutive menstrual periods may be missed. The effect is presumably the result of arrest of maturation of the Graafian follicles, but there appears to be no permanent damage to ovarian function.

Local reactions to extravasation of mechlorethamine into the subcutaneous tissue result in a severe, brawny, tender induration that may persist for a long time. If the local reaction is unusually severe, a slough may result. If it is obvious that extravasation has occurred, the involved area should be promptly infiltrated with an isotonic solution of sodium thiosulfate (⅙ M); an ice compress then should be applied intermittently for 6 to 12 hours. The purpose of the thiosulfate is to provide an ion that reacts avidly with the nitrogen mustard and thereby protects tissue constituents. If thiosulfate solution is not available, prompt injections of isotonic sodium chloride solution may have some value by reducing the local concentration of the vesicant agent.

Thrombophlebitis is a potential complication of therapy with mechlorethamine. It rarely occurs if the drug is injected into the tubing during the course of an intravenous infusion.

CYCLOPHOSPHAMIDE

Efforts to modify the chemical structure of mechlorethamine to achieve greater selectivity for neoplastic tissues led to the development of cyclophosphamide. After studies of the pharmacological activity of cyclophosphamide, clinical investigations by European workers demonstrated its effectiveness in selected malignant neoplasms. (For references to the original literature, *see* Calabresi and Welch, 1962; Symposium, 1967.) The chemical structure of cyclophosphamide and the interesting rationale that led to its synthesis have been presented above (*see* Table 55–1).

Pharmacological and Cytotoxic Actions. None of the severe acute CNS manifestations reported with the typical nitrogen mustards has been noted with cyclophosphamide. Nausea and vomiting, however, may occur. Although the general cytotoxic action of this drug is similar to that of other alkylating agents, some notable differences have been observed. When compared with mechlorethamine, damage to the megakaryocytes and thrombocytopenia are less common. Another unusual manifestation of selectivity consists in more prominent

damage to the hair follicles, resulting frequently in alopecia. The drug is not a vesicant, and local irritation does not occur.

Absorption, Fate, and Excretion. Cyclophosphamide is well absorbed orally. As mentioned above, the drug is activated by metabolism in the liver by the mixed-function oxidase system of the smooth endoplasmic reticulum (Brock, 1967); several toxic metabolites have been identified (Colvin, 1982). The current view of the metabolism and fate of cyclophosphamide is presented in Figure 55–3. The hepatic cytochrome P-450 mixed-function oxidase system converts cyclophosphamide to 4-hydroxycyclophosphamide, which is in a steady state with the acyclic tautomer, aldophosphamide. These compounds may be oxidized further by hepatic aldehyde oxidase and perhaps by other enzymes, yielding the metabolites carboxyphosphamide and 4-ketocyclophosphamide, neither of which possesses significant biological activity. It appears that hepatic damage is minimized by these secondary reactions, whereas significant amounts of the activated metabolites, such as aldophosphamide, are transported to the target sites by the circulatory system. It has been proposed that, in cells that are susceptible to cytolysis, the aldophosphamide is cleaved by a β-elimination reaction, generating stoichiometric amounts of phosphoramide mustard and acrolein, both of which are highly cytotoxic. In addition, nor-nitrogen mustard has been identified in the plasma of patients treated with cyclophosphamide. It is not known which of the active metabolites (*e.g.*, phosphoramide mustard, 4-hydroxycyclophosphamide, or nor-nitrogen mustard) plays the key role in the therapeutic and toxic actions of cyclophosphamide. Acrolein may be responsible for the hemorrhagic cystitis seen during therapy with cyclophosphamide. This can be reduced in intensity or prevented by the parenteral administration of N-acetylcysteine or other sulfhydryl compounds; acrolein reacts readily with sulfhydryl groups (Colvin, 1982).

If the cytochrome P-450 system is induced by pretreatment of an animal with phenobarbital or inhibited by administration of proadifen (SK & F 525-A), however, the antitumor activity and therapeutic index of cyclophosphamide are not significantly modified (Sladek, 1972). The explanation proposed for this unexpected finding illustrates several important pharmacological principles. Cy-

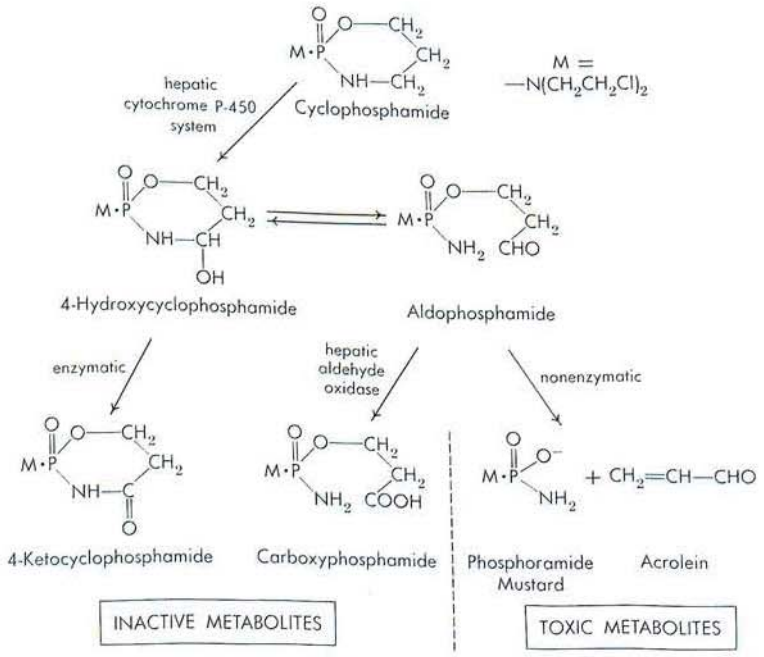


Figure 55–3. *Metabolism of cyclophosphamide.*

clophosphamide, which is biologically relatively inactive, is eliminated from the body very slowly. The activated metabolites (*e.g.*, aldophosphamide) alkylate the target sites in susceptible cells in an "all-or-none" type of reaction or are detoxicated by formation of inactive metabolites that are rapidly excreted by the kidneys. The cytotoxic effects are related to the total amount rather than to the velocity of generation of the activated metabolites. Thus, it seems likely that the biological actions of cyclophosphamide may be affected more drastically by alterations in the rates of detoxication and elimination than by changes in the rate of generation of the activated metabolites.

Urinary and fecal recovery of unchanged cyclophosphamide is minimal after intravenous administration. Maximal concentrations in plasma are achieved 1 hour after oral administration, and a significant amount of unchanged drug is found in the stool when this route is employed. The half-life of cyclophosphamide in plasma is about 7 hours (Bagley *et al.*, 1973). Prior treatment with allopurinol significantly prolongs this value.

Preparations, Dosage, and Routes of Administration. *Cyclophosphamide* (CYTOXAN) is supplied as 25- and 50-mg tablets and as a powder (100 to 2000 mg) in sterile vials. Solutions are prepared by addition of 5 ml of sterile water per 100 mg of drug.

The drug has been administered orally, intravenously, intramuscularly, intrapleurally, and intraperitoneally. A conservative daily dose of 2 to 3 mg/kg, orally or intravenously, has been recommended for patients with more susceptible neoplasms such as lymphomas and leukemias or with compromised bone-marrow function. A higher daily dosage of 4 to 8 mg/kg intravenously for 6 days, followed by an oral maintenance dose of 1 to 5 mg/kg daily, 3 to 5 mg/kg intravenously twice weekly, or 10 to 15 mg/kg intravenously every 7 to 10 days, has been used for the treatment of carcinomas and more resistant neoplasms. Large single doses of 30 mg/kg (750 to 1000 mg/sq m) have been very effective in patients with lymphomas and cause a rapid response approaching that seen with mechlorethamine; in patients without complications or previous therapy, the recommended total initial loading dose is 40 to 50 mg/kg (1500 to 1800 mg/sq m), administered intravenously over a period of 2 to 5 days. Careful evaluation of bone-marrow function is imperative, and prolonged therapy is guided by keeping the total leukocyte count between 2500 and 4000 cells per cubic millimeter of blood or by obtaining the desired response of the tumor.

Therapeutic Uses and Clinical Toxicity. The clinical spectrum of activity for cyclophosphamide is very broad and similar to that of nitrogen mustard. It is an essential component of many effective drug combinations. The drug is effective in Hodgkin's disease and other lymphomas. Complete remissions and presumed cures have been reported in Burkitt's lymphoma and in acute lymphoblastic leukemia of childhood when cyclophosphamide is used concurrently with other agents. It is frequently used in combination with methotrexate and fluorouracil as adjuvant therapy after surgery for carcinoma of the breast when there is involvement of axillary nodes (Bonadonna and Valagussa, 1983).

Notable advantages of this drug are the availability of oral as well as parenteral routes of administration and the possibility of giving fractionated doses over prolonged periods of time. For these reasons it possesses a versatility of action that allows an intermediate range of use, between that of the highly reactive intravenous mechlorethamine and that of oral chlorambucil. Beneficial results have been obtained in multiple myeloma; chronic lymphocytic leukemia; carcinomas of the lung, breast, cervix, and ovary; as well as in neuroblastoma, retinoblastoma, and other neoplasms of childhood. In addition to the combination mentioned above, it is also often used in combination with doxorubicin, vincristine, and prednisone. (*See* Holland and Frei, 1982.)

Because of its potent *immunosuppressive* properties, cyclophosphamide has received considerable attention for the control of organ rejection after transplantation and in nonneoplastic disorders associated with altered immune reactivity, including Wegener's granulomatosis, rheumatoid arthritis, the nephrotic syndrome in children, and autoallergic ocular disease. Appropriate caution is advised when the drug is considered for use in these conditions, not only because of its acute toxic effects but also because of its high potential for inducing sterility, teratogenic effects, mutations, and cancer. The drug should not be used during pregnancy or breast feeding. (*See* Kaplan and Calabresi, 1973; Gershwin *et al.*, 1974; Calabresi, 1979.)

The clinical toxicity of cyclophosphamide differs from that of other nitrogen mustards in that significant degrees of thrombocytopenia are much less common, but there is frequent occurrence of alopecia. Patients should be forewarned of this possible event, which is usually reversible even without interruption of therapy. Nausea and vomiting are common and occur with equal frequency whether the drug is given by the oral or the intravenous route. Mucosal ulcerations, dizziness of short duration, transverse ridging of the nails, increased skin pigmentation, interstitial pulmonary fibrosis, and hepatic toxicity have been reported. Extravasation of the drug into subcutaneous tissues does not produce local reactions, and thrombophlebitis does not complicate intravenous administration. The occurrence of sterile, hemorrhagic cystitis has been reported in 5 to 10% of patients. This has been attributed to chemical irritation produced by reactive metabolites of cyclophosphamide. Its incidence has been reduced by administration of N-acetylcysteine (*see* above). For routine clinical use, ample fluid intake and frequent voiding are recom-

mended. Administration of the drug should be interrupted at the first indication of dysuria or hematuria. The syndrome of inappropriate secretion of antidiuretic hormone (ADH) has been observed in patients receiving cyclophosphamide, usually at doses higher than 50 mg/kg (DeFronzo *et al.*, 1973). It is important to be aware of the possibility of water intoxication, since these patients are usually vigorously hydrated.

Melphalan

This phenylalanine derivative of nitrogen mustard is also known as L-sarcolysin. Early clinical studies demonstrated a spectrum of activity similar to that of other alkylating agents.

Chemistry. The chemical structure and the rationale for the synthesis of this amino acid derivative of mechlorethamine have been presented above (*see* Table 55–1; *see also* Colvin, 1982; Wheeler, 1982; Vistica, 1983).

Pharmacological and Cytotoxic Actions. The general pharmacological and cytotoxic actions of melphalan are similar to those of other nitrogen mustards. The drug is not a vesicant.

Absorption, Fate, and Excretion. When given orally, melphalan is absorbed in an incomplete and variable manner, and 20 to 50% of the drug is recovered in the stool. The drug has a half-life in plasma of approximately 90 minutes, and 10 to 15% of an administered dose is excreted unchanged in the urine (Tattersall *et al.*, 1978; Alberts *et al.*, 1979b; Colvin, 1982).

Preparation, Dosage, and Route of Administration. *Melphalan* (ALKERAN) is available in scored, 2-mg tablets. The usual oral dose is 6 mg daily for a period of 2 to 3 weeks, during which time the blood count should be carefully followed. A rest period of up to 4 weeks should then intervene. When the leukocyte and platelet counts are rising, maintenance therapy, ordinarily 2 to 4 mg daily, is begun. It is usually necessary to maintain a significant degree of bone-marrow depression (total leukocyte count in the range of 3000 to 3500 cells per cubic millimeter) in order to achieve optimal results.

Therapeutic Uses and Clinical Toxicity. Although the general spectrum of action of melphalan seems to resemble that of other nitrogen mustards, the advantages of a gradual but continuous administration by the oral route have made the drug useful in the treatment of *multiple myeloma* (Bergsagel, 1972). Beneficial effects have also been reported in malignant melanoma and in carcinoma of the breast and ovary. The clinical toxicity of melphalan is mostly hematological and is similar to that of other alkylating agents. Nausea and vomiting are infrequent. Alopecia does not occur, and changes in renal or hepatic function have not been observed.

Uracil Mustard

Uracil mustard was synthesized in an unsuccessful attempt to produce an active-site-directed alkylator by linking the *bis*-(2-chloroethyl) group to the pyrimidine base uracil. Its activity in experimental neoplasms was demonstrated shortly thereafter. No relationship has been demonstrated, however, with the biological functions of uracil.

Chemistry. The structural formula of uracil mustard and its chemical relationship to other alkylating agents are presented above (*see* Table 55–1). The compound is quite unstable in water.

Pharmacological and Cytotoxic Actions. Uracil mustard may cause nausea and vomiting. The drug is not a vesicant. Cytotoxicity characteristic of the nitrogen mustards has been observed in subacute and chronic toxicity studies of uracil mustard in animals.

Absorption, Fate, and Excretion. Uracil mustard is absorbed quickly but not completely after oral administration in dogs. Concentrations in plasma decline rapidly after either oral (2 mg/kg) or intravenous (1 mg/kg) administration, and no evidence of drug is detected at 2 hours. Less than 1% of the administered dose is recovered unchanged in the urine.

Preparation, Dosage, and Route of Administration. *Uracil mustard* is available in capsules containing 1 mg. Two oral dosage schedules are recommended: (1) 1 to 2 mg daily for 3 weeks, repeated after an interruption of 1 week; and (2) 3 to 5 mg daily for 7 days, then 1 mg daily for 3 weeks.

Therapeutic Uses and Clinical Toxicity. Uracil mustard can be administered orally and, in contrast to cyclophosphamide, does not cause frank alopecia. Its clinical spectrum of action is similar to that of other related alkylating agents. Hematopoietic depression is the major manifestation of toxicity, and uracil mustard has been considered useful for controlling thrombocytosis. Nausea, vomiting, diarrhea, and dermatitis have also been noted.

Chlorambucil

Initial clinical studies of this aromatic derivative of mechlorethamine demonstrated beneficial results primarily in chronic lymphocytic leukemia, as well as in Hodgkin's disease and related malignant lymphomas. (For references to the early reports, *see* Calabresi and Welch, 1962.)

Chemistry. The chemical formula of chlorambucil and its relation to the nitrogen mustards are presented above (*see* Table 55–1).

Pharmacological and Cytotoxic Actions. Although CNS side effects can occur, these have been observed only with large doses. Nausea and vomiting may result from single oral doses of 20 mg or more. Cytotoxic effects on the bone marrow, lymphoid organs, and epithelial tissues are similar to those observed with the nitrogen mustards.

Absorption, Fate, and Excretion. Oral absorption of chlorambucil is adequate and reliable. The

drug has a half-life in plasma of approximately 1 hour, and it is almost completely metabolized (Alberts *et al.*, 1979a).

Preparation, Dosage, and Route of Administration. *Chlorambucil* (LEUKERAN) is available in 2-mg tablets for oral administration. The standard initial daily dosage is 0.1 to 0.2 mg/kg, continued for at least 3 to 6 weeks. The total daily dose, usually 4 to 10 mg, is given at one time. With fall in the peripheral total leukocyte count or clinical improvement, the dosage is reduced; maintenance therapy (usually 2 mg daily) is feasible and may be required, depending on the nature of the disease.

Therapeutic Uses and Clinical Toxicity. At the recommended dosages, chlorambucil is the slowest-acting nitrogen mustard in clinical use. It is the treatment of choice in chronic lymphocytic leukemia and in primary (Waldenström's) macroglobulinemia.

In chronic lymphocytic leukemia, chlorambucil may be given orally for long periods of time, achieving its effects gradually and often without toxicity to a precariously compromised bone marrow. Its spectrum of action is similar to that of other alkylating agents, and remissions may be expected in Hodgkin's disease and lymphomas, and sometimes in solid tumors. Clinical improvement comparable to that with melphalan or cyclophosphamide has been observed in some patients with plasma-cell myeloma. Beneficial results have also been reported in disorders with altered immune reactivity, such as vasculitis associated with rheumatoid arthritis and autoimmune hemolytic anemia with cold agglutinins (Livingston and Carter, 1970; Gardner, 1972; Knospe *et al.*, 1974).

Although it is possible to induce marked hypoplasia of the bone marrow with excessive doses of chlorambucil administered over long periods of time, its myelosuppressive action is usually moderate, gradual, and rapidly reversible. Gastrointestinal discomfort, azoospermia, amenorrhea, pulmonary fibrosis, seizures, dermatitis, and hepatotoxicity may be encountered. A marked increase in the incidence of leukemia and other tumors has been noted in a large controlled study of its use for the treatment of polycythemia vera by the National Polycythemia Vera Study Group, as well as in patients with breast cancer receiving long-term adjuvant chemotherapy (Lerner, 1978).

ETHYLENIMINES AND METHYLMELAMINES

TRIETHYLENEMELAMINE (TEM), THIOTEPA (TRIETHYLENETHIOPHOSPHORAMIDE), AND HEXAMETHYLMELAMINE (HMM)

Chemistry. The chemical structures of TEM, thiotepa, and HMM are discussed above in conjunction with the structure-activity relationship of the alkylating agents.

Status. Although still available for clinical use, ethylenimines are now seldom employed as therapeutic agents, having been replaced by selected nitrogen mustards. Their pharmacological properties are described in the *third edition* of this textbook.

Although hexamethylmelamine (HMM) was introduced for clinical trial in the early 1960s, it has recently been shown that the drug has significant activity in small-cell carcinoma of the lung, ovarian cancer, breast carcinoma, and lymphomas, some of which are resistant to alkylating agents. Hexamethylmelamine is available only for investigational purposes in the United States. It is administered orally but is poorly absorbed, and it causes nausea and vomiting, myelosuppression, and neurotoxicity. A more soluble compound, pentamethylmelamine, which is a major metabolite of HMM, has antitumor activity and is under clinical investigation (Chabner, 1982d).

ALKYL SULFONATES

BUSULFAN

During the course of an investigation to determine the antineoplastic properties of a series of alkanesulfonic acid esters, the rather selective action of *busulfan* was detected. This finding led to the use of the drug in patients with chronic granulocytic leukemia. The chemical formula of busulfan is shown on page 1252.

Pharmacological and Cytotoxic Actions. Busulfan is unique in that it exerts virtually no pharmacological action other than myelosuppression. At low doses, selective depression of granulocytopoiesis is evident. Platelets are also affected by relatively small amounts of drug, and erythroid elements may be suppressed as the dosage is raised; eventually, a pancytopenia results. Cytotoxic action does not appear to extend to either the lymphoid tissues or the gastrointestinal epithelium.

Absorption, Fate, and Excretion. Busulfan is well absorbed after oral administration, and it disappears from the blood with a half-time of 2 to 3 hours. Almost all of the drug is excreted in the urine as methanesulfonic acid. The metabolism of busulfan has been reviewed in relation to its mechanism of action by Warwick (1963).

Preparation, Dosage, and Route of Administration. *Busulfan* (MYLERAN) is available in scored, 2-mg tablets. The initial oral dose varies with the total leukocyte count and the severity of the disease; daily doses from 2 to 8 mg are recommended to initiate therapy and are adjusted appropriately to

subsequent hematological and clinical responses. It has been reported that reduction of the total leukocyte count to 10,000 or fewer cells per cubic millimeter before discontinuing the drug results in longer remissions. If maintenance doses are required to keep the hematological status under control, 1 to 3 mg may be given daily.

Therapeutic Uses and Clinical Toxicity. The beneficial effects of busulfan in chronic granulocytic leukemia are well established, and remissions may be expected in 85 to 90% of patients after the initial course of therapy.

Reduction in morbidity is readily apparent with symptomatic response, characterized by increased appetite and sense of well-being, which may occur within a few days. Reduction of the leukocyte count is noted during the second or third week, and regression of splenomegaly follows. Beneficial results have been reported in other myeloproliferative disorders, including polycythemia vera and myelofibrosis with myeloid metaplasia. The drug is of no value in acute leukemia or in the "blastic crisis" of chronic granulocytic leukemia.

The major toxic effects of busulfan are related to its myelosuppressive properties, and thrombocytopenia may be a hazard. Occasional instances of nausea, vomiting, diarrhea, impotence, sterility, amenorrhea, and fetal malformation have been reported. The drug may be carcinogenic and leukemogenic (Stott *et al.*, 1977). Hyperuricemia, resulting from extensive purine catabolism accompanying the rapid cellular destruction, and renal damage from precipitation of urates have been noted. To avoid this complication, the concurrent use of *allopurinol* is recommended. A number of unusual complications have been observed in patients receiving busulfan, but their relation to the drug is poorly understood; these include generalized skin pigmentation, cataracts, gynecomastia, cheilosis, glossitis, anhidrosis, and pulmonary and endocardial fibrosis (Calabresi and Welch, 1962; Colvin, 1982).

NITROSOUREAS

The nitrosoureas are important antitumor agents that have demonstrated activity against a wide spectrum of human malignancies; they appear to function chemotherapeutically as bifunctional alkylating agents. Since their introduction by investigators at the Southern Research Institute (Johnston *et al.*, 1963; Schabel, 1973), many nitrosoureas have been synthesized. Certain of these agents, particularly carmustine (BCNU) and lomustine (CCNU), have attracted special interest because of their high lipophilicity and, thus, their capacity to cross the blood-brain barrier; this enables their use in the treatment of meningeal leukemias and brain tumors. Unfortunately, the nitrosoureas used in the clinic to date, with the exception of streptozocin, cause profound, cumulative myelosuppression that restricts their therapeutic value. In addition, long-term treatment with the nitrosoureas, especially semustine (methyl-CCNU), has resulted in renal failure with lesions that resemble radiation-induced nephritis. As with other alkylating agents, the nitrosoureas are both carcinogenic and mutagenic (Colvin, 1982).

Streptozocin, originally discovered as an antibiotic, is of special interest. This compound has a methylnitrosourea (MNU) moiety attached to the 2 carbon of glucose (*see* Table 55–2). It has special affinity for beta cells of the islets of Langerhans and is employed as a diabetogenic agent in experimental animals. Streptozocin is useful in the treatment of human pancreatic islet-cell carcinoma and malignant carcinoid tumors, as well as other human malignancies (Schein *et al.*, 1973, 1974). Although MNU, the active moiety of streptozocin, is cytotoxic to selected human tumors, it also produces powerful and delayed myelosuppression. Furthermore, MNU is particularly prone to cause carbamoylation of lysine residues of proteins (Figure 55–2). Streptozocin is not myelosuppressive and displays little carbamoylating activity. Thus, the nitrosourea-type moiety has been attached to various carrier molecules, with alterations in crucial properties such as tissue specificity, distribution, and toxicity. Chlorozotocin, an agent in which the 2 carbon of glucose is substituted by the chloronitrosourea group (CNU), was developed and subjected to clinical testing (Green *et al.*, 1982). This compound, unlike streptozocin, is not diabetogenic and, unlike many other nitrosoureas, causes little myelosuppression or carbamoylation. There is reason for optimism that important new nitrosourea-containing derivatives will be prepared (Colvin, 1982; Wheeler, 1982).

CARMUSTINE (BCNU)

This compound was the first of the nitrosourea series to receive extensive clinical evaluation. It is effective against a wide range of experimental tumors.

Pharmacological and Cytotoxic Actions. Carmustine is capable of inhibiting the synthesis of DNA, RNA, and protein in a manner similar but not identical to that of other alkylating agents (Livingston and Carter, 1970). Although bone-marrow suppression is observed, there is an unusually delayed onset of leukopenia and thrombocytopenia that is characteristic of this drug. The nadir of the leukocyte and platelet counts may not be reached until 6 weeks after treatment. Cytotoxic effects on the liver, kidneys, and CNS have been reported (Oliverio, 1973).

Absorption, Fate, and Excretion. Although carmustine is rapidly absorbed by the oral route, it is administered intravenously because tissue uptake and metabolism occur quickly; disappearance from the plasma takes place with a half-life of 90 minutes. Approximately 80% of radioactively labeled drug appears in the urine within 24 hours as degradation products of the parent compound. The pharmacokinetic properties of the drug may be affected by the lipid content of the plasma and the other tissues. Active metabolites may be responsible for the delayed bone-marrow toxicity. Entry of these products into the cerebrospinal fluid (CSF) is rapid, and their concentrations in the CSF of man are 15 to 30% of the concurrent plasma values (Oliverio, 1973, 1976; Levin *et al.*, 1978).

Preparation, Dosage, and Route of Administration. *Carmustine* (BICNU) is a powder at 4° C; it melts to an oily liquid at 27° C and is stable in the anhydrous state. It is available in vials containing 100 mg. The half-life of the drug in 0.9% sodium chloride solution at pH 6 is 24 hours at room temperature. Carmustine is usually administered intravenously at doses of 100 to 200 mg/sq m, given by infusion during a period of 1 to 2 hours, and it is not repeated for 6 weeks. When used in combination with other chemotherapeutic agents, the dose is usually reduced by 25 to 50%.

Therapeutic Uses and Clinical Toxicity. The spectrum of activity of carmustine is similar to that of other alkylating agents, with significant responses observed in Hodgkin's disease and to a lesser extent in other lymphomas and myeloma. Because of its ability to cross the blood-brain barrier, it has been used in meningeal leukemia and in primary and metastatic tumors of the brain, with encouraging results. Beneficial responses have been reported in melanomas, as well as in gastrointestinal, breast, bronchogenic, and renal-cell carcinomas (Young *et al.*, 1971; Carter, 1973; Moertel, 1973; Walker, 1973; Wilson *et al.*, 1976).

The most significant clinical toxicity is the characteristically delayed hematopoietic depression described above. The drug is not a vesicant, but local burning pain has been reported after intravenous administration. Nausea and vomiting occur approximately 2 hours after injection, and flushing of the skin and conjunctiva, CNS toxicity, esophagitis, diarrhea, dyspnea, interstitial pulmonary fibrosis, and renal and hepatic toxicity have been reported (Young *et al.*, 1971; Durant *et al.*, 1979; Wiemann and Calabresi, 1985).

LOMUSTINE (CCNU) AND SEMUSTINE (METHYL-CCNU)

Pharmacological and Cytotoxic Actions. Lomustine and its methylated analog, semustine, were selected for clinical studies because of their lipid solubility and superiority to carmustine in the treatment of certain experimental tumors. The cytotoxic effects of these compounds are similar to those of carmustine, as is their clinical toxicity. Delayed bone-marrow depression, reflected by leukopenia and thrombocytopenia, is characteristic and similar to that caused by carmustine (Moertel, 1973; Wasserman *et al.*, 1975; Wasserman, 1976).

Absorption, Fate, and Excretion. Lomustine and semustine are rapidly absorbed from the gastrointestinal tract and are administered orally. Although lomustine is rapidly and completely metabolized, prolonged plasma half-life of its metabolites, ranging from 16 to 48 hours, has been reported. Approximately 50% of the administered dose is detectable in the urine within 24 hours and 75% within 4 days. Radioactively labeled semustine is not detectable in either plasma or urine. The chloroethyl moiety has a half-life of 36 hours, while the cyclohexyl portion has a biphasic disappearance curve with an early half-life of 24 hours and a slower secondary phase with a half-life of 72 hours. Although neither drug can be detected intact in the CSF, active

metabolites appear in significant concentrations within 30 minutes (Oliverio, 1973, 1976; Carter and Slavik, 1974).

Preparations, Dosage, and Route of Administration. *Lomustine* (CEENU) is available in 100-mg, 40-mg, and 10-mg capsules. Semustine is available only for investigational use. The usual oral dose of lomustine is 130 mg/sq m, while the recommended oral dose of semustine is 200 mg/sq m. Both drugs are administered as a single dose, which is not repeated for 6 weeks. When used concurrently with other antineoplastic drugs, the dose is usually reduced by 25 to 50% (Wasserman, 1976).

Therapeutic Uses and Clinical Toxicity. These agents have a wide spectrum of activity. Lomustine appears to be more effective than carmustine in Hodgkin's disease. Beneficial results of therapy with lomustine and particularly semustine, alone and concurrently with other agents, have been reported in patients with malignant gliomas, adenocarcinomas of the gastrointestinal tract, Hodgkin's disease and other lymphomas, carcinoma of the breast, malignant melanoma, hypernephromas, multiple myeloma, and various squamous-cell carcinomas (Symposium, 1973; Carter and Slavik, 1974; Wilson *et al.*, 1976; Moertel, 1978).

The clinical toxicity of both drugs is similar, with the characteristically delayed bone-marrow suppression described above being the dose-limiting effect. Nausea and vomiting are frequently encountered. Nephrotoxicity may occur, particularly when semustine is administered at doses greater than 1500 mg/sq m. The earliest manifestation of this toxic effect may be a decrease in renal size. Both drugs are mutagenic, carcinogenic, and leukemogenic (Green *et al.*, 1982; Calabresi, 1983).

STREPTOZOCIN

This naturally occurring nitrosourea is an antibiotic derived from *Streptomyces acromogenes*. It has been particularly useful in treating functional, malignant pancreatic islet-cell tumors. The drug is capable of inhibiting synthesis of DNA in microorganisms and mammalian cells; it affects all stages of the mammalian cell cycle. Biochemical studies have also revealed potent inhibitory effects on pyridine nucleotides and on key enzymes involved in glyconeogenesis.

Absorption, Fate, and Excretion. Streptozocin is administered parenterally. After intravenous infusions of 200 to 1600 mg/sq m, peak concentrations in the plasma are 30 to 40 μg/ml; the half-life of the drug is approximately 15 minutes. Only 10 to 20% of a dose is recovered in the urine (Schein *et al.*, 1973).

Preparation, Dosage, and Route of Administration. *Streptozocin* (ZANOSAR) is available in 1-g vials as a powder for injection. The intravenous dose is 500 mg/sq m once daily for 5 days; this course is repeated every 6 weeks. Alternatively, 1000 mg/sq m can be given weekly for 2 weeks, and the weekly dose can then be increased to a maximum of 1500 mg/sq m.

Therapeutic Uses and Clinical Toxicity. Streptozocin has been used primarily in patients with metastatic pancreatic islet-cell carcinoma, and beneficial responses are translated into a significant increase in 1-year survival rate and a doubling of median survival time for the responders. It has also been found to be active in Hodgkin's disease, other lymphomas, and occasionally in melanoma and malignant carcinoid tumors (Schein *et al.*, 1974). Broder and Carter (1973) noted nausea and vomiting in almost all of 52 patients treated for islet-cell carcinoma. Renal or hepatic toxicity occurs in approximately two thirds of cases; although usually reversible, renal toxicity may be fatal, and proximal tubular damage is the most important toxic effect. Serial determinations of urinary protein are most valuable in detecting early renal effects. Hematological toxicity, consisting in anemia, leukopenia, or thrombocytopenia, occurs in 20% of patients.

TRIAZENES

DACARBAZINE (DTIC)

Dacarbazine, the chemistry of which is described above, was originally believed to act as an antimetabolite; more recent evidence indicates that it functions as an alkylating agent after metabolic activation in the liver by microsomal enzymes. Dacarbazine appears to inhibit the synthesis of RNA and protein more than that of DNA. It kills cells slowly, and there appears to be no phase of the cell cycle in which sensitivity is increased (Bono, 1976). Minimal immunosuppressive activity has been noted in mice but not clinically (Chabner, 1982d).

Absorption, Fate, and Excretion. Dacarbazine is administered intravenously; after an initial rapid phase of disappearance ($t_{1/2}$ of about 20 minutes), the drug is removed from plasma with a half-time of about 5 hours (Loo *et al.*, 1976). The half-life is prolonged in the presence of hepatic or renal disease. Almost one half of the compound is excreted intact in the urine by tubular secretion. Elevated urinary concentrations of 5-aminoimidazole-4-carboxamide (AIC) are derived from the catabolism of dacarbazine, rather than by inhibition of *de-novo* purine biosynthesis. Concentrations of dacarbazine in CSF are approximately 14% of those in plasma (Chabner, 1982d).

Preparation, Dosage, and Route of Administration. *Dacarbazine* (DTIC-DOME) is available in vials that contain 100 or 200 mg. The recommended regimen is to give 3.5 mg/kg per day, intravenously, for a 10-day period; this is repeated every 28 days. Alternatively, 250 mg/sq m can be given daily for 5 days and repeated every 3 weeks. Extravasation of the drug may cause tissue damage and severe pain.

Therapeutic Uses and Clinical Toxicity. At present, dacarbazine is employed principally for the treatment of malignant melanoma; the overall response rate is about 20%. Beneficial responses have also been reported in patients with Hodgkin's disease, particularly when the drug is used concurrently with doxorubicin, bleomycin, and vinblastine (Santora and Bonadonna, 1979), as well as in various sarcomas when used with doxorubicin (Costanzi, 1976; Gottlieb *et al.*, 1976). Toxicity includes nausea and vomiting in more than 90% of patients; this usually develops 1 to 3 hours after treatment. Myelosuppression, with both leukopenia and thrombocytopenia, is usually mild to moderate. A flulike syndrome, consisting in chills, fever, malaise, and myalgias, may occur during treatment. Hepatotoxicity, alopecia, facial flushing, neurotoxicity, and dermatological reactions have also been reported.

II. Antimetabolites

FOLIC ACID ANALOGS

METHOTREXATE

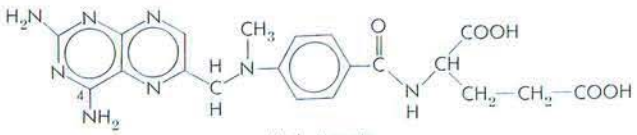
This class of antimetabolites not only produced the first striking, although temporary, remissions in leukemia (Farber *et al.*, 1948) but also includes the first drug to achieve cures of choriocarcinoma in women (Hertz, 1963). The attainment of a high percentage of permanent remissions in this otherwise-lethal disease provided great impetus to chemotherapeutic investigation. Interest in folate antagonists has increased greatly with the introduction of "rescue" technics that employ leucovorin (folinic acid, citrovorum factor) and/or thymidine to protect normal tissues against lethal damage. These methods permit the use of very high doses of folate analogs such as methotrexate and extend their utility to tumors such as osteogenic sarcoma that do not respond to lower doses.

Methotrexate has also been used with benefit in the therapy of *psoriasis*, a nonneoplastic disease of the skin characterized by abnormally rapid proliferation of epidermal cells (McDonald, 1981). Additionally, folate antagonists are potent inhibitors of some types of immune reactions and have been employed as *immunosuppressive agents*, for example, in organ transplantation. (For recent reviews, *see* Symposium, 1981b; Chabner, 1982c; Johns and Bertino, 1982; Jackson, 1984.)

Structure-Activity Relationship. Folic acid is an essential dietary factor from which is derived a coenzyme, tetrahydrofolic acid, and a group of structurally related derivatives; these are concerned with the metabolic transfer of one-carbon units. A detailed description of the biological functions and therapeutic applications of folic acid appears in Chapter 57.

Although there are many metabolic loci where folate analogs (antifols) might act, the enzyme dihydrofolate reductase (DHFR) is the primary site of action of most analogs studied to date (*see* Figure 57–1). This enzyme has been purified from a number of species. Important structural differences among the various enzymes have enabled the design of important therapeutic agents for the treatment of bacterial and malarial infections (*see* discussion of trimethoprim, Chapter 49; pyrimethamine, Chapter 45). These inhibitors have much greater activity against the bacterial and protozoal DHFRs than they do against the mammalian enzyme. Such developments have introduced a new level of sophistication into the science of chemotherapy and suggest the possibility of developing new analogs of folate that have unique advantages for the chemotherapy of neoplastic diseases.

Because folic acid and many of its analogs are very polar, they cross the blood-brain barrier poorly and require specific transport mechanisms to enter mammalian cells. Once in the cell, additional glutamyl residues are added to the molecule by the enzyme folylpolyglutamate synthetase. Intracellular methotrexate polyglutamates have been identified with as many as five glutamyl residues. Since these polyglutamates cross cellular membranes poorly, if at all, this serves as a mechanism of entrapment and may account for the prolonged retention of methotrexate in tissues such as liver. Evidence indicates that polyglutamylated folates have substantially greater affinity than the monoglutamate form for enzymes such as thymidylate synthetase. Other findings indicate that distinct differences exist in the folate influx system in certain tumors in comparison with normal tissues (*e.g.*, bone marrow). Novel folate antagonists have been devised to attempt to exploit these differences. The analog 10-deaza,10-ethyl aminopterin is transported into many tumor cells much more



Methotrexate

efficiently than into normal tissues, is polyglutamylated, and is an excellent inhibitor of DHFR. This promising new compound will be evaluated clinically in the near future (Sirotnak, 1983). In efforts to bypass the obligatory membrane transport system and facilitate penetration of the blood-brain barrier, a number of lipid-soluble folate antagonists have been synthesized; several of these are in the early stages of clinical trial (Johns and Bertino, 1982; Jackson 1984). (For recent reviews, *see* Chabner, 1982c; Goldman *et al.*, 1983; Hitchings, 1983; Jolivet and Chabner, 1983; McGuire *et al.*, 1983; Sirotnak, 1983; Cadman, 1984; Jackson, 1984.)

Mechanism of Action. To understand the mechanism of action of folate analogs such as methotrexate, it is necessary to appreciate the complexities of the metabolism of folate cofactors and their multiplicity of functions; this is discussed in Chapter 57. To function as a cofactor in one-carbon transfer reactions, folate must first be reduced by DHFR to tetrahydrofolate (FH_4). Single-carbon fragments are added enzymatically to FH_4 in various configurations and may then be transferred in specific synthetic reactions. A key metabolic event is catalyzed by thymidylate synthetase and involves the conversion of 2-deoxyuridylate (dUMP) to thymidylate, an essential component of DNA. The methyl group transferred to the uracil moiety of dUMP is donated by $N^{5,10}$-methylene FH_4. Significantly, this carbon atom is transferred to the pyrimidine ring at the oxidation level of formaldehyde and is reduced to methyl by the pteridine ring of the folate coenzyme; the result is the formation of dihydrofolate (FH_2). Thus, to function again as a cofactor, FH_2 must first be reduced to FH_4 by DHFR. Inhibitors with a high affinity for DHFR prevent the formation of FH_4 and cause major disruptions in cellular metabolism by producing an acute intracellular deficiency of folate coenzymes. The folate coenzymes become trapped as FH_2 polyglutamates, which cannot function metabolically. One-carbon transfer reactions crucial for the *de-novo* synthesis of purine nucleotides and of thymidylate cease, with the subsequent interruption of the synthesis of DNA and RNA (as well as other vital metabolic reactions).

Understanding of these events enables appreciation of the rationale for the use of thymidine and/or leucovorin (N^5-formyl FH_4; folinic acid) in the "rescue" of normal cells from toxicity caused by drugs such as methotrexate. Leucovorin is a fully reduced, metabolically functional folate coenzyme; it enters cells via the specific carrier-mediated transport system and is convertible to other folate cofactors. Thus, it may function directly, without the need for reduction by DHFR in reactions such as those required for purine biosynthesis. On the other hand, thymidine may be converted to thymidylate by thymidine kinase, thus bypassing the reaction catalyzed by thymidylate synthetase and providing the necessary precursor for DNA synthesis.

An important feature of the binding of active folate antagonists with DHFRs is the very low inhibition constants observed (on the order of 1 nM). Covalent bonds are not involved in the enzyme-inhibitor interactions despite the high affinity of the antagonists for the protein. Substantial progress has been made in defining the chemical basis for the binding of methotrexate to DHFR (*see* Matthews *et al.*, 1978; Chabner, 1982c).

As with most inhibitors of cellular reproduction, a selective effect on neoplastic cells is obtainable to only a partial extent with methotrexate. Folate antagonists kill cells during the S phase of the cell cycle, and evidence indicates that methotrexate is much more effective when the cellular population is in the logarithmic phase of growth, rather than in the plateau phase. Because it is also capable of inhibiting RNA and protein synthesis, however, methotrexate slows the entry of cells into S phase and its cytotoxic action has been referred to as "self-limiting" (Skipper and Schabel, 1982).

Mechanism of Resistance to Antifolates. Although evidence is incomplete, three biochemical mechanisms of acquired resistance to methotrexate have been clearly demonstrated: (1) impaired transport of methotrexate into cells, (2) production of altered forms of DHFR that have decreased affinity for the inhibitor, and (3) increased concentrations of intracellular DHFR. It has been known for years that blood elements with marked increases in the activity of DHFR appear within days after treatment of patients with leukemia with single doses of methotrexate. This may reflect induction of new enzyme synthesis, temporary elimination from the marrow of cells that are susceptible to the drug because of low enzymatic activity, or protection of DHFR against catabolic degradation by intracellular proteases. It is well established that the enzyme, complexed with methotrexate, undergoes conformational changes that render it remarkably resistant to proteolysis.

Of special interest is the phenomenon of gene amplification and its relationship to acquired resistance to methotrexate and, perhaps, other cytotoxic agents. Methotrexate-resistant cell lines have been isolated that have several hundredfold more DHFR than do wild type cells because of comparable increases in the mRNA specific for the enzyme. This is due to the occurrence in these resistant cells of increased numbers of copies of the gene for DHFR. (For further discussion, *see* Schimke *et al.*, 1978; Bertino *et al.*, 1983; Stark and Wahl, 1984.)

Various therapeutic tactics have been recommended to avoid selection of resistant cells. The use of high doses of methotrexate with leucovorin "rescue" may permit the intracellular accumulation of methotrexate in concentrations that inactivate DHFR even when the enzyme is present at markedly elevated levels. Alternation of treatment with methotrexate with other active therapeutic agents that function by different mechanisms is another way to attempt to kill cells that are resistant.

General Toxicity and Cytotoxic Action. The actions of 4-amino analogs of folate in animals have been studied extensively.

Animals given a minimal lethal dose survive for at least 48 hours and usually die within 3 to 5 days. Anorexia, progressive weight loss, bloody diarrhea, leukopenia, depression, and coma are the outstanding features of fatal intoxication. The major lesions occur in the *intestinal tract* and *bone marrow*. Swelling and cytoplasmic vacuolization of the mucosal cells of the intestinal epithelium are evident within 6 hours. These changes are followed by desquamation of epithelial cells, extrusion of plasma into the lumen of the bowel, and leukocytic infiltration of the submucosa. Terminally, the entire intestinal tract exhibits a severe hemorrhagic desquamating enteritis. Degeneration of bone marrow develops rapidly. Within 24 hours there is evident disturbance in the maturation of erythrocytes. Proliferation of erythroid precursors is inhibited, and significant proportions of primitive erythroid elements have the appearance of megaloblasts. Rapid pathological alteration in myelopoiesis also occurs, and within a few days the bone marrow becomes aplastic. There is diminution in content of lymphoid cells in lymphatic tissue, but there is no evidence of necrosis. The disturbance in hematopoiesis is reflected in the circulating blood by a marked granulocytopenia and reticulocytopenia and a moderate lymphopenia.

Folic acid antagonists seriously interfere with *embryogenesis*. The site of action is on the embryonic mesenchyme. Decidual and placental tissues are unaffected by doses of the drugs that cause fetal death. Young embryos are much more susceptible than are the more developed. The administration of methotrexate during pregnancy obviously is accompanied by great hazards to the fetus.

Absorption, Fate, and Excretion. Methotrexate is readily absorbed from the gastrointestinal tract at doses routinely employed in clinical practice (0.1 mg/kg), but larger doses are incompletely absorbed. The drug is also absorbed from parenteral sites of injection. Peak concentrations in the plasma of 1 to 10 μM are obtained after doses of 25 to 100 mg/sq m, and concentrations of 0.1 to 1 mM are achieved after high-dose infusions of 1.5 g/sq m or more (Chabner, 1982c). A direct relationship exists between dose and plasma concentrations. Following intravenous administration, the drug disappears from plasma in a triphasic fashion (Huffman *et al.*, 1973). The first phase, due to the distribution into body fluids, has a half-time of about 45 minutes. The second phase reflects renal clearance ($t_{1/2}$ of about 2 hours). The final phase has a half-time of approximately 7 hours and begins when the concentration in plasma approximates 0.1 μM. This terminal half-life, if unduly prolonged, may be responsible for major toxic effects of the drug on the marrow and gastrointestinal tract. Distribution of methotrexate into body spaces, such as the pleural or peritoneal cavities, may occur. If such spaces are expanded (*e.g.*, by ascites or pleural effusion), they may act as a site of storage and release of drug with resultant prolonged elevation of plasma concentrations and more severe toxicity.

Approximately 50% of the drug is bound to plasma proteins. Laboratory studies suggest that it may be displaced from plasma albumin by a number of drugs, including sulfonamides, salicylates, tetracycline, chloramphenicol, and phenytoin; caution should be used if these are given concomitantly. Of the drug absorbed, from 40 to 50% of a small dose (2.5 to 15 μg/kg) to about 90% of a larger dose (150 μg/kg) is excreted unchanged in the urine within 48 hours, mostly within the first 8 hours. A small amount of methotrexate is also excreted in the stool, probably through the biliary tract. Metabolism of methotrexate in man does not seem to occur to a significant degree. After high doses, however, metabolites do accumulate; these include a potentially nephrotoxic 7-hydroxylated compound (*see* Chabner, 1982c). The portion of each dose of methotrexate that normally is excreted rapidly gains access to the urine by a combination of glomerular filtration and active tubular secretion. Therefore, the concurrent use of drugs that also undergo tubular secretion, as well as impaired renal function, can influence markedly the response to this drug. Particular caution must be exercised in treating patients with renal insufficiency.

The portion of methotrexate that is retained in human tissues remains for long periods, for example, for weeks in the kidneys and for several months in the liver.

The drug is converted to polyglutamates in hepatocytes, and there is also evidence for enterohepatic recirculation (Chabner *et al.*, 1981).

It is important to emphasize that methotrexate is very poorly transported across the blood-brain barrier; hence, neoplastic cells that have entered the CNS probably are not affected by usual concentrations of drug in the plasma. When high doses of methotrexate are given, followed by leucovorin "rescue" (*see* below), substantial concentrations of methotrexate may be attained in the CNS. The pharmacokinetic properties of methotrexate have been discussed by Goldin (1978); *see also* Appendix II.

Preparations, Dosage, and Routes of Administration. *Methotrexate* (*amethopterin*; FOLEX, MEXATE) is provided in scored, 2.5-mg tablets and also as a dry powder (the sodium salt) in vials containing 20 to 250 mg for preparation of sterile injectable solutions.

Although the standard daily oral dosage of methotrexate ordinarily employed in patients with leukemia has been 2.5 to 5 mg for children and 2.5 to 10 mg for adults, newer therapeutic concepts have emerged involving revised dosage schedules and the use of multiple drugs sequentially and concurrently. Methotrexate induces remission slowly, probably because the cells in advanced leukemia are not in the logarithmic phase of growth. For induction of remission it has been superseded by the more rapid and effective therapy with vincristine plus prednisone, with or without daunorubicin. Methotrexate is of great value in the maintenance of remissions, particularly when administered intermittently at doses of 30 mg/sq m, intramuscularly, twice a week, or by intensive 2-day "pulses" of 175 to 525 mg/sq m at monthly intervals.

The intrathecal administration of methotrexate has been employed, particularly when manifestations of cerebral involvement in either leukemia or choriocarcinoma have appeared, as occurs not infrequently even during systemic remissions. This route of administration achieves high concentrations of methotrexate in the CSF and is effective also in patients whose systemic disease has become resistant to methotrexate, since the leukemic cells in the CNS beyond the blood-brain barrier have survived in a pharmacological sanctuary and retain their original degree of sensitivity to the drug. The recommended intrathecal dose is 0.2 to 0.5 mg/kg, given once or repeated at intervals of 2 to 5 days, depending on the severity of involvement and the response to therapy; another dosage schedule is 12 mg/sq m once weekly for 2 weeks and then monthly. Leucovorin may be administered intramuscularly to counteract the systemic toxicity of methotrexate.

In the treatment of choriocarcinoma with methotrexate, 15 mg/sq m (15 to 30 mg) is administered daily for 5 days orally or parenterally. Courses are repeated at 1- to 2-week intervals, toxicity permitting, and urinary gonadotropin titers are used as a guide for persistence of disease.

Methotrexate has been used in the treatment of severe, disabling *psoriasis* in doses of 2.5 mg orally for 5 days, followed by a rest period of at least 2 days, or 10 to 25 mg intravenously weekly. An initial parenteral test dose of 5 to 10 mg is recommended to detect any possible idiosyncrasy. Complete awareness of the pharmacology and toxic potential of methotrexate is a prerequisite for its use in this nonneoplastic disorder (Weinstein, 1977).

Continuous infusion of relatively large amounts of methotrexate may be employed (from 250 mg to 1 g/sq m, or more, weekly), but only when the technic of leucovorin "rescue" is used. The rationale for the administration of high doses is to achieve an excess of intracellular unbound drug, such that DNA synthesis is inhibited almost completely. Extremely high (0.1 to 1 mM) concentrations of drug must be achieved extracellularly in order to overcome any deficiency of the carrier-mediated transport system. After infusion of methotrexate for 6 hours, leucovorin is injected at a dose of 6 to 15 mg/sq m every 6 hours for 72 hours; the goal is to rescue normal cells and thereby prevent toxicity. The administration of methotrexate in high dosages may be extremely dangerous and should be performed only by experienced chemotherapists who are capable of quantification of the concentrations of methotrexate and leucovorin in plasma. With appropriate precautions, these investigational schedules are surprisingly free of toxicity. It is imperative to maintain the output of a large volume of alkaline urine, since methotrexate precipitates in the renal tubules in acidic urine. In the presence of malignant effusions, delayed clearance may cause severe toxicity. Although the use of methotrexate in high doses with leucovorin "rescue" has been studied clinically for several years with very encouraging results, the optimal timing, dose of leucovorin required, and proof of enhanced therapeutic efficacy remain to be established (*see* Goldin, 1978; Symposium, 1981b; Chabner, 1982c).

Therapeutic Uses and Clinical Toxicity. Methotrexate is a useful drug in the management of *acute lymphoblastic leukemia* in children. However, methotrexate is of very limited value in the types of leukemia seen in adults. It is of established value in *choriocarcinoma* and related trophoblastic tumors of women, with complete and lasting remissions occurring in approximately 75% of women treated sequentially with methotrexate and dactinomycin, and in over 90% when early diagnosis is accompanied by a low concentration of gonadotropin in the urine. A number of these patients are living without evidence of disease more than 25 years after initiation of therapy. In addition, many women with nonmetastatic trophoblastic disease, hydatidiform mole, and chorioadenoma destruens have been treated successfully with methotrexate. Beneficial results have also been reported in patients with

mycosis fungoides, Burkitt's and other non-Hodgkin's lymphomas, and carcinomas of the breast, tongue, pharynx, bladder, and testes (in conjunction with chlorambucil and dactinomycin), as well as in occasional patients with other tumors. High-dose methotrexate, with subsequent leucovorin "rescue," can cause substantial tumor regression in at least two tumors highly refractory to most chemotherapeutic agents: carcinoma of the lung and osteogenic sarcoma. (For references, *see* Symposium, 1981b; Chabner, 1982c; Calabresi *et al.*, 1985.) Striking improvement has been observed with the use of methotrexate in the treatment of severe psoriasis. Furthermore, methotrexate is an effective immunosuppressive agent and has been used for prevention of graft-versus-host reactions that result from marrow transplantation, as well as in the management of dermatomyositis, rheumatoid arthritis, Wegener's granulomatosis, and pityriasis rubra pilaris (*see* Weinstein, 1977; Goldin, 1978).

Treatment with methotrexate requires constant surveillance of the patient in order to judge dosage properly and to avoid serious toxic reactions. In persons treated with conventional doses or with concomitant leucovorin, it is frequently possible to avoid severe leukopenia or aplasia of the bone marrow. Thrombocytopenia with bleeding can be treated with platelet transfusions, but it may be difficult to control, particularly in the presence of infection. It is imperative that a skilled medical team and sophisticated facilities, particularly abundant platelet transfusions and measures for preventing and combating infections, be available in order to provide the intensive supportive therapy necessary to control the severe toxic manifestations that may result when intensive dosage schedules are used.

Other untoward reactions also may complicate the use of methotrexate (Wiemann and Calabresi, 1985). Ulcerative stomatitis and diarrhea are frequent side effects and require interruption of the therapeutic regimen; hemorrhagic enteritis and death from intestinal perforation may occur. Additional toxic manifestations include alopecia, dermatitis, interstitial pneumonitis, neurotoxicity, nephrotoxicity, defective oogenesis or spermatogenesis, abortion, teratogenesis, and hepatic dysfunction, usually reversible but sometimes leading to cirrhosis. The long-term complications associated with the use of methotrexate for immunosuppressive therapy are discussed by Schein and Winokur (1975).

PYRIMIDINE ANALOGS

This class of agents encompasses a diverse and interesting group of drugs that have in common the capacity to impede the biosynthesis of pyrimidine nucleotides or to mimic these natural metabolites to such an extent that they interfere with vital cellular activities, such as the synthesis and functioning of nucleic acids. Certain of the drugs in this group are employed in the treatment of a variety of afflictions, including neoplastic diseases, psoriasis, and infections caused by fungi and DNA-containing viruses. When selected members of the group are used together or concurrently with other antimetabolites, synergistic effects have been demonstrated against various experimental tumors, and some of these treatment schedules are being investigated clinically. (*See* reviews by Maley, 1977; Chabner, 1982e.)

General Mechanism of Action. The antineoplastic agents fluorouracil (5-FU) and cytarabine (AraC), the antiviral compound idoxuridine, and the antifungal agent flucytosine (Chapter 54) are the drugs in this group that are established clinically. Other compounds are under clinical investigation, as are potentially synergistic combinations of pyrimidine analogs and other types of inhibitors.

Among the best-characterized agents in this class are the halogenated pyrimidines, a group that includes such compounds as fluorouracil and idoxuridine. If one compares the van der Waals radii of the various substituents (Table 55–3), the dimension of the fluorine atom resembles that of hydrogen, whereas the bromine and iodine atoms are close in size to the methyl group. Idoxuridine has relatively little effect on the biosynthesis of thymidylic acid; like thymidine, however, it is converted enzymatically within cells to phosphorylated derivatives; it is also degraded to the corresponding base, iodouracil, which is converted to uracil and iodide. The phosphorylated forms of idoxuridine inhibit competitively the utilization of the analogous derivatives of thymidine and can lead, in appropriate circumstances, to incorporation of the analog, as iododeoxyuridylic acid, into DNA in place of thymidylic acid. These activities can suppress temporarily the growth of both experimental and human neoplasms; in addition, incorporation of the iodo- or bromo- analogs into DNA renders the latter more susceptible to the injurious effects of radiation.

If the hydrogen on position 5 of the pyrimidine ring is replaced with fluorine, the chemical reactivity of the ring is significantly altered, although the molecule, fluorouracil, behaves as does uracil with several enzymes. Fluorine has an inductive (electron-withdrawing) effect, which is reflected in a much lower pK_a with fluorouracil-containing compounds than with the natural compounds. The ionization that occurs is as follows:

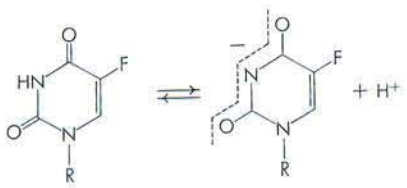
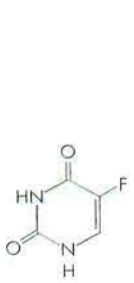
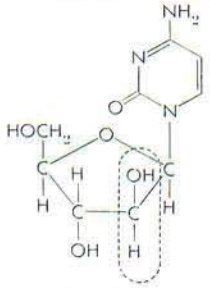


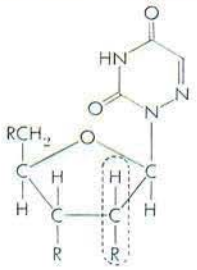
Table 55–3. STRUCTURAL FORMULAS OF PYRIMIDINE ANALOGS



Fluorouracil
(pK_a 8.1)

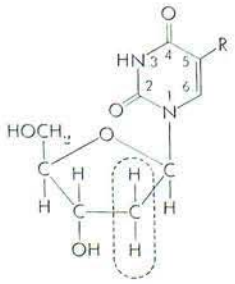


Cytarabine
(Cytosine Arabinoside)
(pK_a 4.5)



Azauridine: R = —OH

Azaribine: R = —O—C(=O)—CH_3
(pK_a 6.7)



R	*van der Waals Radii (Å)*	*Compound*	pK_a
H	1.20	Deoxyuridine	9.3
F	1.35	Floxuridine (fluorodeoxyuridine)	7.6
Cl	1.80	Chlorodeoxyuridine	7.9
Br	1.95	Bromodeoxyuridine	7.9
CH_3	2.00	Thymidine	9.8
I	2.15	Idoxuridine (iododeoxyuridine)	8.25
CF_3	2.44	Trifluoromethyldeoxyuridine	7.35

In addition, the carbon-fluorine bond is stronger than the carbon-hydrogen bond and is less susceptible to enzymatic cleavage. Thus, substitution of a halogen atom of the correct dimensions can produce a molecule that sufficiently resembles a natural pyrimidine to interact with enzymes of pyrimidine metabolism and also to interfere drastically with certain other aspects of pyrimidine action.

Among the various modifications of the sugar moiety attempted, the replacement of the ribose of cytidine with arabinose has yielded a useful chemotherapeutic agent, cytarabine. As may be seen in Table 55–3, the deviation from normal in this case involves the 2′ carbon of the pentose, in which the hydroxyl group is in the opposite configuration from that of the natural ribonucleoside, cytidine. This yields a molecule that sufficiently resembles a deoxynucleoside to be capable of conversion to the nucleotide level, but which blocks the synthesis of DNA (*see* reviews by Chabner, 1982e; Heidelberger, 1982; Kufe and Major, 1982; Pallavicini, 1984).

Several agents are available that inhibit different steps in the synthesis of pyrimidines and that exert synergistic cytotoxicity when used concurrently with a pyrimidine analog such as cytosine arabinoside or 5-fluorouracil. N-phosphonoacetyl-L-aspartate (PALA) is a "transition-state" inhibitor of the enzyme aspartate transcarbamylase, which catalyzes an early step in pyrimidine biosynthesis (Stark and Bartlett, 1983). Two other agents are potent inhibitors of a later step in pyrimidine nucleotide synthesis, the coupled enzymatic reactions by which orotate is converted first to the 5′-monophosphate nucleotide (orotidylate) and then decarboxylated to form uridylate. The compounds 6-azauridine and pyrazofuran, after being converted to the corresponding 5′-monophosphate nucleotides, are potent inhibitors of orotidylate decarboxylase. Two other agents, 3-deazauridine and the glutamine antagonist acivicin, block the conversion of uridine triphosphate (UTP) to cytidine triphosphate (CTP). Use of these various agents in combination with cytosine arabinoside has resulted in increased formation of intracellular AraCTP and synergistic cytotoxicity. Recognition that the sugar phosphate, 5-phosphoribosyl-1-pyrophosphate (PRPP), is an essential metabolite for the biosynthesis of both purines and pyrimidines has suggested other synergistic drug combinations. When *de-novo* purine biosynthesis is inhibited by an agent such as methotrexate, the steady-state intracellular concentrations of PRPP rise. This enhances considerably the rate of the lethal synthesis of 5-fluorouridylate from 5-fluorouracil, a reaction that utilizes PRPP. This has led to the clinical trial of timed administrations of methotrexate followed by 5-fluorouracil (Cadman, 1984).

Fluorouracil and Floxuridine (Fluorodeoxyuridine)

The chemistry of these analogs is discussed above.

Mechanism of Action. Fluorouracil, as such, is without significant inhibitory activity in mammalian systems, and, in order to inhibit cellular growth, it must first be converted enzymatically to the nucleotide level. Several routes are available for the formation of the 5′-monophosphate nucleotide (F-UMP) in animal cells. Fluorouracil may be converted to fluorouridine by uridine phosphorylase and then to F-UMP by uridine kinase or it may react directly with PRPP, catalyzed by the enzyme orotate phosphoribosyl transferase, to form F-UMP. The latter enzyme is present in higher concentrations in certain tumors than in liver and reacts with orotate as its natural substrate. Many metabolic pathways are available to F-UMP, including incorporation into RNA. A reaction sequence crucial for antineoplastic activity involves reduction of the diphosphate nucleotide by the enzyme ribonucleotide diphosphate reductase to the deoxynucleotide level and the eventual formation of 5-fluoro-2′-deoxyuridine-5′-phosphate (F-dUMP). This complex metabolic pathway for the generation of the actual growth inhibitor, F-dUMP, may be bypassed through use of the deoxyribonucleoside of fluorouracil—floxuridine (fluorodeoxyuridine, FUdR)—which is a substrate for intracellular thymidine kinase. Thus, in a single enzymatic step, the inhibitor of thymidylate synthetase, F-dUMP, can be produced in cells by the use of FUdR. Unfortunately, FUdR is a good substrate for both thymidine and uridine phosphorylases, and it is rapidly degraded to fluorouracil.

There have been notable advances in our understanding of the interaction between F-dUMP and the enzyme thymidylate synthetase, which is an important site of the cytotoxic action of the drug. The folate cofactor, $N^{5,10}$-methylenetetrahydrofolate, and F-dUMP form a covalently bound ternary complex with the enzyme, which resembles the transition state formed during the normal enzymatic reaction when dUMP is converted to thymidylate. The stable complex inactivates the enzyme. (For details of this mechanism and the nature of the postulated inhibitory ternary complex, *see* Danenberg and Lockshin, 1981.)

Fluorouracil is incorporated into both RNA and DNA. Incorporation into RNA has been associated with toxicity and has major effects on both the processing and functions of RNA. While the incorporation of FUdR into DNA has been described, its significance is unclear.

Although it has been shown that fluorouracil is much more lethal to logarithmically growing cells than to stationary cells, there is no clearly demonstrated effect at a definite stage of the cell cycle. The phenomenon of "thymineless death" has been invoked to explain the cytotoxic effects of fluorouracil and its derivatives. The blockade of the thymidylate synthetase reaction inhibits DNA synthesis, while cellular production of both RNA and protein continues. An imbalance in growth occurs that is not compatible with cell survival. In accord with this proposal, the administration of thymidine can often reverse the toxicity, presumably through bypass of the block at thymidylate synthetase.

A number of biochemical mechanisms have been identified that are associated with resistance to the cytotoxic effects of fluorouracil or floxuridine. These mechanisms include loss or decreased activity of the enzymes necessary for activation of fluorouracil, decreased pyrimidine monophosphate kinase (which decreases incorporation into RNA), and acquisition of an altered thymidylate synthetase that is not inhibited by F-dUMP. Unfortunately it is not established which (if any) of these mechanisms is associated with inherent or acquired resistance to fluorouracil and its derivatives that is encountered clinically. (For reviews, *see* Danenberg and Lockshin, 1981; Chabner, 1982e; Heidelberger, 1982; Heidelberger *et al.*, 1983; Valeriote and Santelli, 1984.)

General Toxicity and Cytotoxic Action. The major sites of action of fluorouracil and floxuridine on normal tissues are the bone marrow and the epithelium of the gastrointestinal and oral mucosa. These are described in detail under Therapeutic Uses and Clinical Toxicity (*see* below).

Absorption, Fate, and Excretion. Fluorouracil and floxuridine are usually administered parenterally, since absorption after ingestion of the drugs is unpredictable and incomplete. Metabolic degradation occurs, particularly in the liver. Floxuridine is converted by thymidine or deoxyuridine phosphorylases into fluorouracil, and the latter is catabolized in much the same way as is uracil. Thus, 5-fluoro-5,6-dihydrouracil is formed, the ring of which is opened to give α-fluoro-β-ureidopropionic acid, which may be degraded further to α-fluoro-β-alanine (Heidelberger, 1975). In man, an important product of the metabolism of fluorouracil is urea.

Rapid intravenous administration of fluorouracil produces plasma concentrations of 0.1 to 1.0 mM; plasma clearance is rapid ($t_{1/2}$ = 10 to 20 minutes). Urinary excretion of intravenously injected fluorouracil-2-^{14}C, given as a single dose, amounts to only 11% in 24 hours; however, during this period, 63% of the radioactivity is expired as carbon dioxide. Given by continuous intravenous infusion for 24 hours, plasma concentrations in the range of 0.5 to 3.0 μM are obtained and the urinary excretion of fluorouracil is only 4%, while the $^{14}CO_2$ excretion rises to 90%. These findings probably account for the lower cytotoxicity of fluorouracil administered by infusion, compared to that seen with single doses. Fluorouracil readily enters the CSF, and concen-

trations of about 7 μM are reached within 30 minutes after intravenous administration; values are sustained for approximately 3 hours and subside slowly during a period of 9 hours (Fraile *et al*., 1980; Chabner, 1982e).

Preparations, Dosage, and Routes of Administration. *Fluorouracil* (*5-FU*; ADRUCIL) is available in sterile ampuls containing 500 mg in 10 ml for intravenous administration. The recommended dose for average-risk patients in good nutritional status with adequate hematopoietic, renal, and hepatic function is 12 mg/kg daily for 4 days, by rapid injection, followed by 6 mg/kg on alternate succeeding days for two to four doses if no toxicity is observed. The maximal daily dose has been established arbitrarily at 800 mg. Treatment should be discontinued at the earliest manifestation of toxicity (usually stomatitis or diarrhea) because the maximal effects of bone-marrow suppression will not be evident until the ninth to fourteenth day. The first course of therapy should be administered either in the hospital or under extremely close supervision in order to establish the tolerance of the individual patient. After a period of 4 weeks from the first injection of the preceding course, a new course of therapy is initiated; the dosage is adjusted on the basis of the previous response and is repeated at monthly intervals. Another type of maintenance schedule is 10 to 15 mg/kg or 500 to 600 mg/sq m, administered weekly as a single rapid injection. It is usually necessary to produce mild-to-moderate toxicity in order to achieve significant antineoplastic effects.

In the selection of patients, the roles of nutritional deficiencies and protein depletion have been stressed, particularly in relation to surgery. Reduced tolerance of the hematopoietic system may be present in elderly patients or as a result of invasion of the bone marrow by either neoplastic cells or myelofibrosis. Patients with compromised bone-marrow function as a result of previous therapy either with alkylating agents or x-ray to the pelvis or vertebrae are particularly sensitive to the myelosuppressive action of these compounds. In patients with extensive liver metastases, catabolism of the drug may be markedly impaired and therapy may be contraindicated; if treatment is instituted, reduced doses must be administered to prevent the hazards of overdosage.

Fluorouracil has been administered by infusion into the hepatic artery with favorable results in patients with metastases to the liver (Ensminger *et al*., 1978).

Topical fluorouracil as a 1 or 5% cream or a 1 to 5% solution in propylene glycol (EFUDEX, FLUOROPLEX) has been used successfully in dermatology.

Floxuridine (*fluorodeoxyuridine;* FUDR) is available for injection as a powder, 500 mg in 5-ml containers. It may be administered in schedules identical with those of fluorouracil, except that the individual doses, in milligrams, are twice those used with the latter agent. Continuous infusion of floxuridine has produced objective responses with 1/30 to 1/60 the dose necessary with multiple individual doses, but with similar toxicity. Continuous infusion of fluorinated pyrimidines into the arterial blood supply of localized tumors, particularly in the liver or in the head and neck region, may provide beneficial clinical effects. Intra-arterial infusions, at doses of 0.1 to 0.6 mg/kg per 24 hours, are administered continuously until local toxicity is encountered (Fraile *et al*., 1980).

Therapeutic Uses and Clinical Toxicity. Clinical use of fluorinated pyrimidines has been concerned primarily with *fluorouracil*, and accumulated experience indicates that the drug can be of palliative value in certain types of carcinoma, particularly of the breast and the gastrointestinal tract; beneficial effects have also been reported in hepatoma, as well as in carcinoma of the ovary, cervix, urinary bladder, prostate, pancreas, and oropharyngeal areas. There is little evidence, however to encourage the expectation that significant overall prolongation of life can be achieved in the majority of patients (Calabresi *et al*., 1985). Fluorouracil is widely used with very favorable results for the topical treatment of premalignant keratoses of the skin and multiple superficial basal-cell carcinomas. It is also effective in severe recalcitrant psoriasis (Alper *et al*., 1985).

The clinical manifestations of toxicity caused by fluorouracil and floxuridine are similar and may be difficult to anticipate because of their delayed appearance. The earliest untoward symptoms during a course of therapy are anorexia and nausea; these are followed shortly after by stomatitis and diarrhea, which constitute reliable warning signs that a sufficient dose has been administered. Stomatitis is manifested by formation of a white patchy membrane that ulcerates and becomes necrotic. The occurrence of similar lesions in the stoma of colostomies and at post-mortem examination of the gastrointestinal tract, as well as complaints of dysphagia, retrosternal burning, and proctitis, indicates that enteric injury may occur at any level. The major toxic effects, however, result from the myelosuppressive action of these drugs; clinically, the effects are most frequently manifested as leukopenia, the nadir of which is usually between the ninth and fourteenth day after the first injection of drug. Thrombocytopenia and anemia may complicate the picture. Loss of hair, occasionally progressing to total alopecia, nail changes, dermatitis, and increased pigmentation and atrophy of the skin may be encountered. Neurological manifestations, including an acute cerebellar syndrome, have been reported, and myelopathy has been observed after

the intrathecal administration of fluorouracil. Cardiac toxicity may also occur. The low therapeutic indices of these agents emphasize the need for very skillful supervision by physicians familiar with the action of the fluorinated pyrimidines and the possible hazards of chemotherapy.

CYTARABINE (CYTOSINE ARABINOSIDE)

Among the more important antimetabolites is cytarabine (1-β-D-arabinofuranosylcytosine; AraC). Its effectiveness in the treatment of acute leukemia is well established. (For reviews, *see* Chabner, 1982b; Kufe and Major, 1982; Pallavicini, 1984.)

Mechanism of Action. This compound is an analog of 2′-deoxycytidine with the 2′-hydroxyl in a position *trans* to the 3′-hydroxyl of the sugar, as shown in Table 55–3. The 2′-hydroxyl causes steric hindrance to the rotation of the pyrimidine base around the nucleosidic bond. The bases of polyarabinonucleotides cannot stack normally as do the bases of polydeoxynucleotides.

As with most purine and pyrimidine antimetabolites, cytarabine must be "activated" by conversion to the 5′-monophosphate nucleotide, in this case catalyzed by deoxycytidine kinase. The nucleotide analog, AraCMP, can react with appropriate nucleotide kinases to form the diphosphate and triphosphate nucleotides (AraCDP and AraCTP). Accumulation of AraCTP causes potent inhibition of DNA synthesis in many cells. Previously, this was thought to result from competitive inhibition of DNA polymerase by AraCTP. However, studies now indicate that inhibition of DNA synthesis by mammalian cells occurs at AraCTP concentrations $\frac{1}{100}$ or less than those required for inhibition of DNA polymerase, and the incorporation of AraC molecules into alkali-labile internucleotide linkages in DNA has been implicated. There is a significant relationship between inhibition of DNA synthesis and the total amount of AraC incorporated into DNA (Kufe and Major, 1982). Thus, the incorporation of about five molecules of AraC per 10^4 bases in DNA decreases cellular clonogenicity by about 50%. There is also evidence that AraC acts by slowing both chain elongation and the movement of newly replicated DNA through the matrix-bound replication apparatus. In addition, AraC inhibits β-DNA polymerase, an enzyme involved in DNA repair. Of interest, AraCTP can inhibit virally induced reverse transcriptase at low concentrations.

AraC shows relatively high cell-cycle specificity and is most cytotoxic to cells in the S phase; it is thus most active against cells that are actively proliferating. Other important cytokinetic effects have been observed. For example, quiescent tumor cells can be recruited into the cell cycle by AraC. Cells exposed to high concentrations of AraC may be arrested at the G_1-S boundary, but, when released from this block, they traverse the cell cycle at an accelerated rate. This increased rate of transit results from shortening of all phases of the cell cycle. Another unexplained effect is the induction of terminal differentiation of certain leukemic cell lines exposed to low concentrations of AraC.

Despite a wealth of observation, the precise mechanism of cellular death caused by AraC is not understood. Potentially important is the phenomenon of "unbalanced growth," which results from prolonged suppression of macromolecular syntheses. Thus, inhibition of DNA synthesis by AraC without concomitant inhibition of protein and RNA syntheses can result in marked increases in cellular volume and in cellular death. It is likely that continued inhibition of DNA synthesis for at least one cell cycle is necessary. This mechanism may thus be important when AraC is administered by continuous prolonged infusion. A number of investigations have indicated that the optimal interval between doses of AraC is about 8 to 12 hours. This interval appears to coincide with the time when a large fraction of the cell population is in S phase (Chabner, 1982b; Kufe and Major, 1982; Pallavicini, 1984).

Mechanisms of Resistance to Cytarabine. Both natural and acquired resistance to AraC are seen. A crucial factor is the relative activities of anabolic and catabolic enzymes that influence the conversion of AraC to AraCTP. The rate-limiting enzyme is deoxycytidine kinase, which produces AraCMP. An important degradative enzyme is deoxycytidine deaminase, which deaminates AraC to the relatively nontoxic metabolite, arauridine. This enzyme is found in high activity in many tissues, including some human tumors. A second degradative enzyme, dCMP deaminase, converts AraCMP to the inactive metabolite, AraUMP. Thus, the balance between the anabolic and catabolic enzymes determines the concentrations of AraCTP achieved. Clear relationships have been shown between the synthesis and retention of high concentrations of AraCTP and the duration of complete remission in patients with acute myeloblastic leukemia (Rustum and Priesler, 1979).

Several biochemical mechanisms have been identified in AraC-resistant subpopulations in various murine and human tumor cell lines. Most commonly encountered are alterations in deoxycytidine kinase. Cells may be wholly or partially deficient in this enzyme or may have an enzyme with altered nucleoside binding affinity. Another mechanism of resistance is marked expansion of the dCTP pool due to increased CTP synthetase activity, with or without a deficiency of dCMP deaminase. The increased concentrations of intracellular dCTP presumably can block the actions of AraCTP on DNA synthesis. Other mechanisms include impaired transport of AraC into cells, increased deoxycytidine deaminase activity, and reduced affinity of DNA polymerase for AraCTP.

Tetrahydrouridine, a relatively potent inhibitor of cytidine deaminase, can enhance net synthesis of AraCTP and increase the cytotoxicity of AraC in several cell lines that have high activities of cytidine deaminase. Unfortunately, when the combination of tetrahydrouridine and AraC was subjected to clinical evaluation, marked increases in myelotoxicity were observed, suggesting that the thera-

peutic index would not be improved. (*See* Chabner, 1982b; Kufe and Major, 1982; Pallavicini, 1984.)

Absorption, Fate, and Excretion. Cytarabine is poorly and unpredictably absorbed after oral administration, with only about 20% of the drug reaching the circulation. Peak concentrations of 2 to 50 μM are measurable in plasma after injection of 30 to 300 mg/sq m intravenously. After intravenous administration the half-time for elimination of cytarabine is about 2.5 hours. Only about 10% of the injected dose is excreted unchanged in the urine within 12 to 24 hours, while 86 to 96% of the radioactivity appears as the inactive, deaminated product, arabinosyl uracil. Higher concentrations of cytarabine are found in CSF after continuous infusion than after rapid intravenous injection. After intrathecal administration of the drug at a dose of 50 mg/sq m, relatively little deamination occurs, even after 7 hours, and peak concentrations of 1 to 2 μM are achieved. The half-life of cytarabine may range from 2 to 11 hours after intrathecal injection (Ho, 1977; Chabner, 1982b; Wiemann and Calabresi, 1985).

Preparation, Routes of Administration, and Dosage. *Cytarabine* (CYTOSAR-U) is marketed as a powder for injection for the treatment of acute leukemias in children and adults. Two dosage schedules are recommended: (1) rapid intravenous injection of 100 to 200 mg/sq m daily for 5 to 7 days; or (2) continuous intravenous infusion of 100 mg/sq m daily for 5 to 7 days. In general, children seem to tolerate higher doses than do adults. *Maintenance* therapy with subcutaneous injections of 1 mg/kg, weekly or every other week, can be used, although the drug appears more effective for the *induction* of remissions in acute leukemia. Intrathecal doses of 30 mg/sq m every 4 days have been used to treat meningeal leukemia.

Therapeutic Uses and Clinical Toxicity. Cytarabine is indicated for induction of remission in acute leukemia in children and adults. When used alone, remission rates of 20 to 40% have been reported. The drug is particularly useful in acute granulocytic leukemia in adults, since chemotherapy is generally disappointing in this disorder. Cytarabine is more effective when used with other agents, particularly thioguanine and daunorubicin; complete remission rates of greater than 50% have been reported. The drug has been studied in patients with a variety of neoplastic diseases. Beneficial effects have been observed in Hodgkin's disease and related lymphomas but very rarely in patients with carcinomas or other tumors. Cytarabine is primarily a potent myelosuppressive agent capable of producing severe leukopenia, thrombocytopenia, and anemia with striking megaloblastic changes. Other toxic manifestations reported include gastrointestinal disturbances and, less frequently, stomatitis, conjunctivitis, hepatic dysfunction, thrombophlebitis at the site of injection, fever, and dermatitis. Seizures and other manifestations of neurotoxicity may occur after intrathecal administration.

AZARIBINE

Azaribine is the triacetyl derivative and prodrug form of azauridine; it was synthesized in order to achieve better absorption following oral administration and to prevent metabolism of azauridine to azauracil by intestinal microorganisms, a factor which contributes to CNS toxicity from azauridine. Azaribine has marked therapeutic activity in psoriasis, mycosis fungoides, and polycythemia vera (McDonald and Calabresi, 1971; Skoda, 1975). Unfortunately, when the drug became available for clinical usage, some patients with psoriasis developed thromboembolic disorders; since evidence indicates an increased incidence of the complication in psoriasis, it is questionable whether this problem should be attributed to the drug or to the disease (McDonald and Calabresi, 1978; Shubin, 1979).

PURINE ANALOGS

Since the pioneering studies of Hitchings and associates, begun in 1942, many analogs of natural purine bases, nucleosides, and nucleotides have been examined in a wide variety of biological and biochemical systems. These extensive investigations have led to the development of several drugs, not only of use in the treatment of malignant diseases (mercaptopurine, thioguanine) but also for immunosuppressive (azathioprine) and antiviral (acyclovir, vidarabine) therapy. The hypoxanthine analog allopurinol, a potent inhibitor of xanthine oxidase, is an important by-product of this effort (*see* Chapter 29). A development of promise has been the discovery of powerful inhibitors of adenosine deaminase, for example, erythrohydroxynonyladenine (EHNA) and pentostatin (2′-deoxycoformycin). In experimental systems these inhibitors of adenosine deaminase have produced marked synergistic effects in combination with various analogs of adenosine, such as vidarabine (arabinosyladenine; AraA); they also show promise

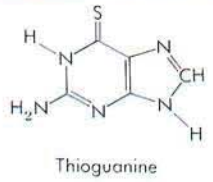
as immunosuppressive agents. (*See* reviews by Elion and Hitchings, 1965; Loo and Nelson, 1982; McCormack and Johns, 1982.)

Structure-Activity Relationship. Mercaptopurine and thioguanine, both established clinical agents for the therapy of human leukemias, are analogs of the natural purines hypoxanthine and guanine, in which the keto group on carbon 6 of the purine ring is replaced by a sulfur atom. Substitution in this position by chlorine or selenium also yields antineoplastic compounds. Cytotoxicity is also observed with the β-D-ribonucleoside and β-D-2′-deoxyribonucleoside derivatives. Because these nucleoside analogs are excellent substrates for purine nucleoside phosphorylase, a highly active enzyme in many tissues, the analog nucleosides often serve as prodrugs and liberate the respective hypoxanthine or guanine analogs in tissues. With several important exceptions, analogs of purine bases or nucleosides must undergo enzymatic conversion to the nucleotide level in order to display cytotoxic activity.

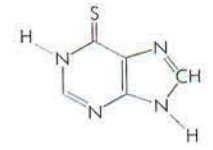
Many attempts have been made to modify the structures of such analogs in order to improve their therapeutic indices or tissue selectivity. Azathioprine (Table 55–4) was developed to decrease the rate of inactivation of 6-mercaptopurine by enzymatic S-methylation, nonenzymatic oxidation, or conversion to thiourate by xanthine oxidase. Azathioprine can react with sulfhydryl compounds such as glutathione (apparently nonenzymatically) and thus serves as a prodrug, permitting the slow liberation of mercaptopurine in tissues. Superior immunosuppressive activity is achieved in comparison with mercaptopurine (Elion, 1967).

An important development has been the discovery of potent inhibitors of adenosine deaminase such as pentostatin (2′-deoxycoformycin; K_i = 2.5 pM) and erythro-9-(2-hydroxy-3-nonyl)-adenine (EHNA; K_i = 2 nM). Pentostatin (Table 55–4) may be viewed as an analog of the natural nucleoside 2′-deoxyinosine, in which the six-membered pyrimidine ring is replaced by a seven-membered diazapin ring. This disrupts the natural aromatic and planar purine ring. The keto-enol tautomer of 2′-deoxyinosine is replaced by a secondary alcohol. These structural changes increase the binding of pentostatin to adenosine deaminase by about 10 million-fold compared to that of adenosine. The enzyme-inhibitor complex dissociates with a $t_{1/2}$ of about 25 to 30 hours (Agarwal *et al.*, 1977; Agarwal, 1982). Thus, pentostatin blocks not only the deamination of natural nucleosides but also that of many analogs used in chemotherapy. Genetic deficiency of adenosine deaminase is associated with malfunction of both T and B lymphocytes, with little effect on other normal tissues (Giblett

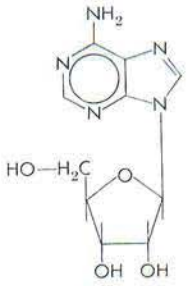
Table 55–4. STRUCTURAL FORMULAS OF ADENOSINE AND VARIOUS PURINE ANALOGS



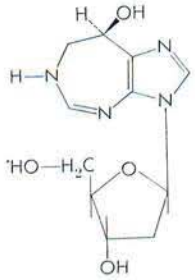
Thioguanine



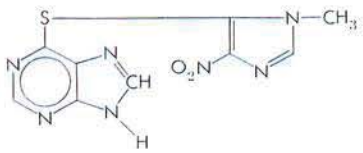
Mercaptopurine



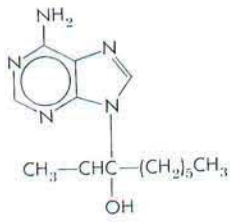
Adenosine



Pentostatin
(Deoxycoformycin)



Azathioprine



Erythrohydroxynonyladenine
(EHNA)

et al., 1972). Thus, animals treated with pentostatin display marked immunosuppression. Synergistic antineoplastic effects are seen when adenosine analogs, such as vidarabine, are administered concurrently with pentostatin. These synergistic effects are striking in rodent tumor systems, and the concurrent administration of pentostatin and analogs of adenosine is under limited clinical trial in patients with advanced cancer. Treatment with pentostatin alone has induced remissions in T-lymphocyte–related diseases, such as T-cell leukemia and mycosis fungoides. Also reported are antineoplastic effects by low doses of pentostatin in B-lymphocyte–related diseases, such as nodular lymphomas, chronic lymphocytic leukemia, and "hairy-cell" leukemia. Unfortunately, a number of unexplained deaths have occurred in phase-I clinical trials of pentostatin, and further extensive study has thus been restricted. (*See* Symposium, 1984; Tritsch, 1985.)

The glutamine antagonists azaserine (O-diazo-acetyl-L-serine), 6-diazo-5-oxo-L-norleucine (DON), and duazomycin, although not purine analogs, are potent inhibitors of the *de-novo* pathway of purine nucleotide biosynthesis. These glutamine analogs are diazoketones with chemical reactivities resembling those of diazomethane. Recently introduced has been the glutamine analog acivicin, which inhibits important enzymes in the pathways for interconversions of both purine and pyrimidine nucleotides, namely CTP synthetase and guanylate synthetase. Also under preclinical study is tiazofurin, an inhibitor of IMP dehydrogenase and thus of the *de-novo* synthesis of GMP. Although these compounds have only weak cytostatic activity when used alone, they can produce significant potentiation when administered with purine or pyrimidine analogs such as mercaptopurine, thioguanine, or AraC.

Mechanism of Action. Although animal tissues have nucleoside kinases that are capable of converting adenosine or the 2′-deoxyribonucleosides of guanine, hypoxanthine, adenine, and many of their analogs to the corresponding 5′-monophosphates, similar reactions do not occur with inosine, guanosine, or their analogs. The latter compounds must first undergo phosphorolysis by purine nucleoside phosphorylase, which is present in high activity in many human tissues. The liberated bases may then be converted to the corresponding nucleotide by hypoxanthine-guanine phosphoribosyltransferase (HGPRT). Similarly, 2′-deoxyguanosine, 2′-deoxyinosine, and many related analogs may react with purine nucleoside phosphorylase, and the product of this reaction, a purine base or analog, is then converted to the corresponding ribonucleoside 5′-monophosphate.

Although a number of biochemical effects of pentostatin have been observed, it is not yet clear why the loss of adenosine deaminase activity (due to a genetic disorder or the presence of an inhibitor) should cause selective lymphotoxicity. Current hypotheses have focused on the consequences of the intracellular accumulation of 2′-deoxyadenosine and of both the extracellular and intracellular accumulation of adenosine in various subpopulations of lymphocytes (*see* Symposium, 1983c). For example, in the presence of pentostatin, 2′-deoxyadenosine is a potent inhibitor of the mitogen-induced proliferation of lymphocytes. Possible underlying mechanisms include the accumulation of dATP (and the consequent inhibition of ribonucleotide reductase) and the profound inhibition of S-adenosylhomocysteine hydrolase by 2′-deoxyadenosine. The latter action leads to an accumulation of S-adenosylhomocysteine and inhibition of various methylation reactions, such as protein carboxymethylation. In addition, various lymphocytic functions (such as lymphocyte-mediated cytolysis) are suppressed by the accumulation of adenosine extracellularly (Zimmerman *et al.*, in Symposium, 1983c). These actions appear to involve enhanced synthesis of adenosine 3′,5′-monophosphate (cyclic AMP), which results from stimulation of receptors for adenosine. In addition, adenosine can cause an increase in the expression of receptors and antigens in T lymphocytes that are associated with suppressor activity and a decreased expression of antigens associated with T-helper/inducer function (Polmar *et al.*, in Symposium, 1983c).

Both thioguanine and mercaptopurine are excellent substrates for HGPRT and are converted to the ribonucleotides 6-thioguanosine-5′-phosphate (6-thioGMP) and 6-thioinosine-5′-phosphate (T-IMP), respectively. Because T-IMP is a poor substrate for guanylate kinase, the enzyme that converts GMP to GDP, T-IMP accumulates intracellularly. Careful studies have demonstrated, however, that mercaptopurine can be incorporated into cellular DNA in the form of thioguanine, indicating that slow reactions catalyzed by enzymes of guanine metabolism can operate. The accumulation of T-IMP may inhibit several vital metabolic reactions: examples are the conversion of inosinate (IMP) to adenylosuccinate (AMPS) and then to adenosine-5′-phosphate (AMP) and the oxidation of IMP to xanthylate (XMP) by inosinate dehydrogenase. These reactions are crucial steps in the conversion of IMP to adenine and guanine nucleotides. On the other hand, in cells incubated with thioguanine, 6-thioGMP first accumulates; it is a poor, but definite, substrate for guanylate kinase. Thus, there is slow conversion to 6-thioGDP and 6-thioGTP and entry of thioguanine nucleotides into the nucleic acids of the cell. In addition, the concentrations of 6-thioGMP achieved are sufficient to cause progressive and irreversible inhibition of inosinate dehydrogenase, presumably through the formation of disulfide bonds. Furthermore, both 6-thioGMP and T-IMP, as well as a number of other 5′-monophosphate derivatives of purine nucleoside analogs, can cause "pseudofeedback inhibition" of the first committed step in the *de-novo* pathway of purine biosynthesis, the reaction of glutamine and PRPP to form ribosylamine-5-phosphate. This enzyme is a major control point in the biosynthesis of purine nucleotides, and its rate of catalysis is highly responsive to the intracellular concentrations of 5′-mononucleotides (natural, as well as analogs). The synthesis of PRPP is also powerfully inhibited by ADP and ATP or related analogs. In view of the

multiplicity of these effects, it can be appreciated that the intracellular accumulation of analogs of various purine nucleotides can produce major metabolic disruptions and, in some instances, may play a key role in cytotoxicity.

Despite extensive investigations, it is still not possible to assess precisely the role of incorporation of thioguanine or mercaptopurine into cellular DNA in the production of either the therapeutic or toxic effects of these drugs. These compounds can cause marked inhibition of the coordinated induction of various enzymes required for DNA synthesis, as well as potentially critical alterations in the synthesis of polyadenylate-containing RNA (Carrico and Sartorelli, 1977).

Other studies indicate that disruption of the synthesis of membrane glycoproteins may be caused by brief exposure to 6-thioguanine. These effects, which are potentially lethal to cellular survival, can occur in model systems at a time before any synthesis of DNA is observed. In view of these diverse biochemical actions, which involve vital systems such as purine biosynthesis, nucleotide interconversions, DNA and RNA synthesis, chromosomal replication, and glycoprotein synthesis, it is not possible to pinpoint a single biochemical event as the cause of thiopurine cytotoxicity. It seems likely that this class of drugs acts by multiple mechanisms (Loo and Nelson, 1982; McCormack and Johns, 1982).

Of many adenosine analogs studied experimentally, *vidarabine* (arabinosyladenine, AraA) has been approved for clinical use in the United States for the treatment of herpetic infections (*see* Chapter 54); its testing as an antineoplastic agent in combination with inhibitors of adenosine deaminase is underway. Vidarabine is converted enzymatically to AraATP. This analog nucleotide can inhibit DNA polymerases by competing with dATP and, in fact, may be incorporated into DNA. In this regard vidarabine resembles the analogous pyrimidine nucleoside antimetabolite cytarabine (AraC). By contrast, vidarabine, when administered alone, is relatively nontoxic and causes minimal immunosuppression. A related compound that has recently been in clinical trial is the analog nucleotide, 2-fluoro-9-β-D-arabinosyladenine-5′-phosphate (2-F-AraAMP), synthesized by Montgomery and Hewson (1969). This analog serves as a prodrug; 2-F-AraA is released by cell membrane–associated 5′-ectonucleotidases. Both 2-F-AraAMP and 2-F-AraA are resistant to enzymatic deamination, which inactivates the parent analog, AraA. Unfortunately, patients who received high intravenous doses of 2-F-AraAMP developed delayed but progressive and fatal CNS toxicity; further clinical study of this agent will likely be curtailed. (For additional discussion and references, *see* Bloch, 1975; Herrmann, 1977; McCormack and Johns, 1982.)

Mechanisms of Resistance to Antipurines. As with other tumor-inhibiting antimetabolites, acquired resistance is a major obstacle to the successful use of antipurines. The most commonly encountered mechanism is deficiency or complete lack of the enzyme HGPRT. In addition, resistance can result from decreases in the affinity of this enzyme for its substrates. Cells that are resistant because of these mechanisms usually show cross-resistance to analogs such as mercaptopurine, thioguanine, and 8-azaguanine.

Another mechanism of resistance identified in cells from leukemic patients is an increase in particulate alkaline phosphatase activity. Other mechanisms include (1) decreased drug transport; (2) increased rates of degradation of the drugs or their intracellular "activated" analogs; (3) alteration in allosteric inhibition of ribosylamine 5-phosphate synthetase; and (4) loss or alterations of the enzymes adenine phosphoribosyltransferase or adenosine kinase (for adenine or adenosine analogs). (For reviews, *see* Brockman, 1974; McCormack and Johns, 1982.)

MERCAPTOPURINE

The introduction of mercaptopurine by Elion and coworkers represents a landmark in the history of antineoplastic and immunosuppressive therapy. Today this antipurine and its derivative, azathioprine, are among the most important and clinically useful drugs of the class. The structure-activity relationship and the mechanism of action and of drug resistance are discussed above. The structural formula of mercaptopurine is presented in Table 55–4.

Absorption, Fate, and Excretion. Absorption of mercaptopurine is incomplete and variable after oral ingestion. About 50% of an oral dose can be accounted for as urinary excretion products in the first 24 hours. After an intravenous dose, the half-life of the drug in plasma is relatively short (about 50 minutes) due to uptake by cells, renal excretion, and rapid metabolic degradation. There are two main pathways for the metabolism of mercaptopurine. The first involves methylation of the sulfhydryl group and subsequent oxidation of the methylated derivatives. The formation of nucleotides of 6-methylmercaptopurine has been shown to occur following administration of mercaptopurine or mercaptopurine ribonucleoside. Substantial amounts of the mono, di, and triphosphate nucleotides of 6-methylmercaptopurine ribonucleoside (6-MMPR) have been identified in the blood and bone marrow of patients treated with mercaptopurine or azathioprine. Desulfuration of thiopurines can occur, and relatively large percentages of the administered sulfur

are excreted as inorganic sulfate. The second major pathway for mercaptopurine metabolism involves the enzyme xanthine oxidase, which is present in relatively large amounts in the liver. Mercaptopurine is a good substrate for this enzyme, which oxidizes it to 6-thiouric acid, a noncarcinostatic metabolite.

An attempt to modify the metabolic inactivation of mercaptopurine by xanthine oxidase led to the development of *allopurinol*. This analog of hypoxanthine is a powerful inhibitor of xanthine oxidase, and not only blocks the conversion of mercaptopurine to 6-thiouric acid but also interferes with the production of uric acid from hypoxanthine and xanthine (*see* Chapter 29). Because of its ability to interfere with the enzymatic oxidation of mercaptopurine and related derivatives, allopurinol increases the exposure of cells to the action of these compounds. Although it greatly potentiates the antineoplastic action of mercaptopurine in tumor-bearing mice, allopurinol increases the toxicity as well, and there is no apparent improvement in the therapeutic index (McCormack and Johns, 1982; Zinner and Klastersky, 1985).

Preparation, Dosage, and Route of Administration. *Mercaptopurine (6-mercaptopurine;* PURINETHOL) is marketed as scored, 50-mg tablets. The initial average daily oral dose is 2.5 mg/kg. Starting doses usually range from 100 to 200 mg a day; with hematological and clinical improvement, the dose is diminished to an appropriate multiple of 25 mg and, in general, maintenance therapy of 1.2 to 2.5 mg/kg a day is continued. If beneficial effects have not been noted after 4 weeks, the dose may be increased gradually until evidence of toxicity is encountered. The total dose required to produce depression of the bone marrow in patients with nonhematological malignancies is about 45 mg/kg and may range from 18 to 106 mg/kg.

Hyperuricemia with hyperuricosuria may occur during treatment; the accumulation of uric acid presumably reflects the destruction of cells with release of purines that are oxidized by xanthine oxidase, as well as an inhibition of the conversion of inosinic acid to precursors of nucleic acids. This circumstance may be an indication for the use of *allopurinol*. Special caution must be employed if mercaptopurine or its imidazolyl derivative, azathioprine, is used with allopurinol, for reasons presented above. Patients treated simultaneously with both drugs should receive approximately 25% of the usual dose of mercaptopurine (*see* Appendix II).

Therapeutic Uses and Clinical Toxicity. In the early studies with mercaptopurine, bone-marrow remissions were described in more than 40% of children with *acute leukemia*. In adults with acute leukemia, the results have been much less impressive, but occasional remissions have been obtained. The drug has contributed to the treatment of lymphoblastic leukemia more by maintaining than by inducing remissions. Cross-resistance does not occur between mercaptopurine and other classes of antileukemic agents.

In the treatment of chronic granulocytic leukemia, maintenance therapy with mercaptopurine can be useful. Mercaptopurine has not been of value in chronic lymphocytic leukemia, Hodgkin's disease and related lymphomas, and a wide variety of carcinomas, even at unusually high doses. Although active as an immunosuppressive agent, it has been superseded by its imidazolyl derivative, azathioprine.

The principal toxic effect of mercaptopurine is bone-marrow depression, although, in general, this develops more gradually than with folic acid antagonists; accordingly, thrombocytopenia, granulocytopenia, or anemia may not be encountered for several weeks. When depression of normal bone-marrow elements occurs, cessation of therapy with the drug usually results in prompt recovery. Anorexia, nausea, or vomiting is seen in approximately 25% of adults, but stomatitis and diarrhea are rare; manifestations of gastrointestinal effects are less frequent in children than in adults. The occurrence of jaundice in about one third of adult patients treated with mercaptopurine has been reported; although the pathogenesis of this manifestation is obscure, it usually clears upon discontinuation of therapy. Its appearance has been associated with bile stasis and hepatic necrosis. Dermatological manifestations have been reported. The long-term complications associated with the use of mercaptopurine and its derivative, azathioprine, for immunosuppressive therapy are discussed by Schein and Winokur (1975).

AZATHIOPRINE

Azathioprine, a derivative of 6-mercaptopurine, is used as an immunosuppressive agent. The structural formula is shown in Table 55–4. The rationale that led to its synthesis and its mechanism of action and metabolic degradation have been discussed above.

Azathioprine (IMURAN) is currently approved for use in the United States only as an adjunct for the prevention of rejection in renal transplantation and for the treatment of severe rheumatoid arthritis. All other uses remain investigational. The drug is available in 50-mg tablets and in vials that contain 100 mg of the sodium salt for injection. The initial oral dose of azathioprine varies from 3 to 5 mg/kg

daily. For maintenance therapy the dose may be reduced to 1 to 3 mg/kg daily, unless rejection is threatened. Patients with transplanted kidneys or impaired renal function may have reduced clearance of the drug and its metabolites; unless the dose is reduced appropriately, a dangerous cumulative effect may result. Among the conditions for which treatment with azathioprine is being studied are idiopathic thrombocytopenic purpura, autoimmune hemolytic anemias, systemic lupus erythematosus, and other disorders believed to be associated with altered immunological reactivity. The drug has been used alone or concomitantly with corticosteroids and other antiproliferative agents. If allopurinol is administered concurrently, the dose of azathioprine should be reduced to approximately 25%, since inhibition of xanthine oxidase impairs the conversion of azathioprine to 6-thiouric acid and may result in dangerous enhancement of its myelosuppressive effect. Bone-marrow depression, usually leukopenia, is the most common toxic effect of azathioprine. Infection may be a complication of any immunosuppressive regimen. Toxic hepatitis and biliary stasis have been reported. Infrequent complications include stomatitis, dermatitis, fever, alopecia, and gastrointestinal disturbances (McCormack and Johns, 1982).

THIOGUANINE

The synthesis of thioguanine was first described by Elion and Hitchings in 1955. It is of particular value in the treatment of acute granulocytic leukemia when given with cytarabine. The structural formula of thioguanine is shown in Table 55–4, and its mechanism of action is discussed above.

Absorption, Fate, and Excretion. Absorption of thioguanine after oral administration is incomplete and erratic. Peak concentrations in the blood are reached 6 to 8 hours after ingestion, and approximately 40% of the dose is excreted in the urine within 24 hours. When thioguanine is administered to man, the S-methylation product, 2-amino-6-methylthiopurine, rather than free thioguanine appears in the urine. After 8 hours, inorganic sulfate becomes a major urinary metabolite. Lesser amounts of 6-thiouric acid are formed, suggesting that deamination catalyzed by the enzyme guanase does not play a major role in the metabolic inactivation of thioguanine. Accordingly, it may be administered concurrently with allopurinol without reduction in dosage, unlike mercaptopurine and azathioprine.

Preparation, Dosage, and Route of Administration. *Thioguanine (6-thioguanine, TG)* is available in scored, 40-mg tablets. The average daily dose is 2 mg/kg. If there is no clinical improvement or toxicity after 4 weeks, the dosage may be cautiously increased to 3 mg/kg daily.

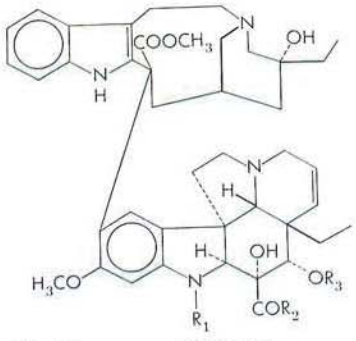
Therapeutic Uses and Clinical Toxicity. Clinically, the compound has been used in the treatment of acute leukemia and, in conjunction with cytarabine, is one of the most effective agents for induction of remissions in acute granulocytic leukemia; it has not been useful in the treatment of patients with solid tumors. Thioguanine has been used as an immunosuppressive agent, particularly in patients with nephrosis and with collagen-vascular disorders. Toxic manifestations include bone-marrow depression and gastrointestinal effects, although the latter may be less pronounced than with mercaptopurine.

III. Natural Products

VINCA ALKALOIDS

History. The beneficial properties of the periwinkle plant (*Vinca rosea* Linn.), a species of myrtle, have been described in medicinal folklore for many years in various parts of the world. While exploring claims that extracts of the periwinkle might have beneficial effects in diabetes mellitus, Noble and coworkers (1958) observed granulocytopenia and bone-marrow suppression in rats, effects that led to purification of an active alkaloid. Other investigations by Johnson and associates demonstrated activity of certain alkaloidal fractions against an acute lymphocytic neoplasm in mice. Fractionation of these extracts yielded four active dimeric alkaloids: *vinblastine, vincristine, vinleurosine,* and *vinrosidine*. Two of these, vinblastine and vincristine, are important clinical agents. Recently introduced is a semisynthetic derivative, vindesine (desacetylvinblastine carboxamide). (*See* Creasey, 1977; Bender and Chabner, 1982; Johnson, 1982; Bender, 1983.)

Chemistry. The vinca alkaloids are very similar chemically. They are asymmetrical dimeric compounds; the structures of vincristine, vinblastine, and vindesine are as follows:



	Vincristine	Vinblastine	Vindesine
R_1:	$-CHO$	$-CH_3$	$-CH_3$
R_2:	$-OCH_3$	$-OCH_3$	$-NH_2$
R_3:	$-COCH_3$	$-COCH_3$	$-H$

No definite information is available regarding metabolic changes that may be necessary for chemical activation or degradative alterations *in vivo*.

Structure-Activity Relationship. Minor differences in structure result in notable differences in toxicity and antitumor spectra among the vinca alkaloids. A number of related dimeric alkaloids are without biological activity. Removal of the acetyl group at C 4 of one portion of vinblastine destroys its antileukemic activity, as does acetylation of the hydroxyl groups. Either hydrogenation of the double bond or reductive formation of carbinols reduces or destroys activity of these compounds.

Mechanism of Action. The vinca alkaloids are cell-cycle–specific agents and, in common with other drugs such as colchicine and podophyllotoxin, block mitosis with metaphase arrest. The biochemical effects of the vinca alkaloids have been explored extensively, and a number of interesting phenomena have been uncovered. It seems likely, however, that most of the biological activities of these drugs can be explained by their ability to bind specifically with the protein tubulin, a key component of cellular microtubules. When cells are incubated with vinblastine, dissolution of the microtubules occurs, and highly regular crystals are formed that contain 1 mole of bound vinblastine per mole of tubulin. Colchicine and podophyllotoxin also can bind specifically with tubulin, but apparently at a site on the protein different from that bound by vinblastine. Through disruption of the microtubules of the mitotic apparatus, cell division is arrested in metaphase. In the absence of an intact mitotic spindle, the chromosomes may disperse throughout the cytoplasm (exploded mitosis) or may occur in unusual groupings, such as balls or stars. The inability to segregate chromosomes correctly during mitosis presumably leads ultimately to cellular death.

In addition to their key role in the formation of mitotic spindles, microtubules have been associated with many other cellular functions. Therefore, it is not surprising that vinca alkaloids may affect these functions as well. Some types of cellular movements, phagocytosis, and certain functions of the CNS appear to involve microtubules, which may explain some of the other effects of vinca alkaloids. (*See* Creasey, 1975.)

Drug Resistance. Despite their structural similarity, a remarkable lack of cross-resistance is seen between the individual vinca alkaloids. Recently, however, attention has been drawn to the phenomenon of pleiotropic drug resistance, in which tumor cells become cross-resistant to a wide range of chemically dissimilar agents. Thus, animal and human tumor cells have been identified that display cross-resistance to vinca alkaloids, the epipodophyllotoxins, anthracyclines, dactinomycin, and colchicine. Chromosomal abnormalities consistent with gene amplification have been observed. Intriguing are reports that calcium channel blockers, such as verapamil, can reverse resistance to vincristine and doxorubicin. (*See* Symposium, 1983a.)

Cytotoxic Actions. Clinical as well as experimental studies have demonstrated that bone-marrow depression, chiefly manifested by leukopenia, is the most important cytotoxic effect on normal cells. In this respect, vincristine is not nearly as potent as vinblastine; with the latter, this is the effect that limits dosage. The relatively low toxicity of vincristine for normal marrow cells makes this agent unusual among antineoplastic drugs, and it is often included in combination chemotherapy with other myelosuppressive agents. Loss of hair, presumably secondary to effects on the epithelial cells of the hair follicles, appears to occur more frequently with vincristine than with vinblastine. No definite explanation is available for the striking differences in the toxicities of these closely related chemical structures.

Neurological Actions. Although neurotoxicity may occasionally be encountered with vinblastine, particularly at high dosage levels, neuromuscular abnormalities are frequently observed with vincristine. Indeed, it is this type of untoward effect that most frequently proves to be the limiting factor during therapy with vincristine. Several types of manifestations have been recognized. In experimental animals, acute toxicity after large doses is characterized by clonic convulsions, muscular weakness, ataxia, tremors, vomiting, and catalepsy. The development of CNS leukemia in patients receiving vincristine and in hematological remission has been interpreted as evidence that the alkaloid penetrates the blood-brain barrier poorly. Although torpor, hallucinations, and coma were observed during exploratory clinical studies with very high doses of vincristine (75 μg/kg weekly), peripheral neuropathy is the most common manifestation of neurotoxicity at usual clinical doses. Numbness and tingling of the extremities, followed by weakness, loss of reflexes, foot-drop, ataxia, muscular cramps, and neuritic pains, have been observed frequently. Clinical neurophysiological studies have demonstrated that asymptomatic depression of the Achilles reflex is the earliest and most consistent sign of vincristine-induced neuropathies. Muscular weakness involving the larynx and the extrinsic muscles of the eye also has been noted. An effect on the autonomic nervous system may be responsible for severe, and even obstructive, constipation that frequently may develop with prolonged administration of vincristine, but it is seen only rarely with vinblastine. Temporary mental depression, occurring on the second or third day after treatment, especially with vinblastine, may be of clinical significance.

Absorption, Fate, and Excretion. Unpredictable absorption has been reported after oral administration of vinblastine, vincristine, or vindesine. At the usual clinical doses the peak concentration of each drug

in plasma is approximately 0.4 μM (Bender and Chabner, 1982). Vinblastine and vincristine bind to plasma proteins. They are extensively concentrated in platelets and to a lesser extent by leukocytes and erythrocytes.

After intravenous injection, vinblastine has a multiphasic pattern of clearance from the plasma; after distribution, drug disappears from plasma with half-lives of approximately 1 and 20 hours (Owellen *et al.*, 1977). Vinblastine is metabolized in the liver to the biologically active derivative desacetylvinblastine. Approximately 15% of an administered dose is detected intact in the urine, and about 10% is recovered in the feces after biliary excretion. Vincristine also has a multiphasic pattern of clearance from the plasma; the terminal half-life is about 2.5 hours (Bender *et al.*, 1977). The drug is metabolized in the liver, but no biologically active derivatives have been identified. Greater toxicity is encountered when vincristine is administered to patients with obstructive jaundice. Less is known about the pharmacokinetics of vindesine; its pattern of disappearance from the plasma resembles that of vinblastine. The major route of elimination of the drug is biliary (Bender and Chabner, 1982).

VINBLASTINE

Preparations, Route of Administration, and Dosage. *Vinblastine sulfate* (VELBAN) is supplied in vials containing 10 mg of dry powder for preparation of solutions (10 ml). The drug is given intravenously; special precautions must be taken against subcutaneous extravasation, since this may cause painful irritation and inflammatory changes. The drug should not be injected into an extremity with impaired circulation. After a single dose of 0.1 to 0.15 mg/kg of body weight, hematological responses are observed for 7 to 10 days. If a moderate level of leukopenia (approximately 3000 cells per cubic millimeter) is not attained, the weekly dose may be increased gradually by increments of 0.05 mg/kg of body weight. Beneficial results, however, may occur at lower doses. Once the optimal amount is established, weekly dosage is continued; if the leukocyte count does not return to 4000 cells per cubic millimeter within 10 to 14 days, the treatment schedule is adjusted accordingly.

Therapeutic Uses and Clinical Toxicity. The most important clinical use of vinblastine is with bleomycin and cisplatin (*see* below) in the therapy of metastatic testicular tumors. This regimen is the preferred treatment for these neoplasms, and a substantial number of complete remissions, which are probably cures, have followed its implementation (Williams and Einhorn, 1985). Beneficial responses have been reported in various lymphomas, particularly Hodgkin's disease, where significant improvement may be noted in 50 to 90% of cases. The effectiveness of vinblastine in a high proportion of lymphomas is not diminished when the disease is refractory to alkylating agents. It is also active in Kaposi's sarcoma, neuroblastoma, and Letterer-Siwe disease (histiocytosis X), as well as in carcinoma of the breast and choriocarcinoma in women.

The nadir of the leukopenia that follows the administration of vinblastine usually occurs within 4 to 10 days, after which recovery ensues within 7 to 14 days; with higher dosage, the total leukocyte counts may not return to normal until 3 weeks have elapsed. Other toxic effects of vinblastine include neurological manifestations as described above. Gastrointestinal disturbances, including nausea, vomiting, anorexia, and diarrhea, may be encountered. The syndrome of inappropriate secretion of antidiuretic hormone (ADH) has been reported, and ischemic cardiac toxicity has also been noted. Loss of hair, mucositis of the mouth, and dermatitis may occur infrequently. Extravasation during injection may lead to cellulitis and phlebitis. Local injection of hyaluronidase and application of moderate heat to the area may be of help by dispersing the drug.

VINCRISTINE

Preparations, Route of Administration, and Dosage. *Vincristine sulfate* (ONCOVIN) is available as a solution in vials containing either 1, 2, or 5 mg of drug. Vincristine used together with corticosteroids is presently the treatment of choice to induce remissions in childhood leukemia; the optimal dosages for these drugs appear to be vincristine, intravenously, 2 mg/sq m of body surface, weekly, and prednisone, orally, 40 mg/sq m, daily. In adults, the usual method of administration is to start therapy with intravenous doses of 0.01 mg/kg of body weight. After observation of the patient for 1 week, the dose is raised by weekly increments of 0.01 mg/kg until either the desired response is obtained or toxicity is encountered. Adult patients with carcinomas or lymphomas often will respond to weekly doses of 0.02 to 0.05 mg/kg. When used with other drugs, for example, in the MOPP regimen (*see* below), the recommended dose of vincristine is 1.4 mg/sq m. High doses of vincristine seem to be tolerated better by children with leukemia than by adults, who may experience severe neurological toxicity. Administration of the drug more frequently than every 7 days or at higher doses seems to increase the toxic manifestations without proportional improvement in the response rate. Maintenance therapy with vincristine is not recommended in children with leukemia (*see* below). Precautions should also be used to avoid extravasation during intravenous administration of vincristine. Vincristine (and vinblastine) can be infused into the arterial blood supply of tumors in doses several times larger than those that can be administered intravenously with comparable toxicity, but inadvertent intrathecal administration of vincris-

tine has been lethal (Gaidys *et al.*, 1983; Williams *et al.*, 1983).

Therapeutic Uses and Clinical Toxicity. Vincristine has a spectrum of clinical activity that is similar to that of vinblastine, but there are some notable differences. An important feature is the lack of cross-resistance between these agents, a remarkable finding in view of the very close similarity of their chemical structures. Vincristine is effective in Hodgkin's disease and other lymphomas. While it appears to be somewhat less beneficial than vinblastine when used alone in Hodgkin's disease, when used with mechlorethamine, prednisone, and procarbazine (the so-called MOPP regimen), it is the preferred treatment for the advanced stages (III and IV) of this disease (DeVita and Hellman, 1982). In non-Hodgkin's lymphomas, vincristine is an important agent, particularly when used with cyclophosphamide, bleomycin, doxorubicin, and prednisone. Vincristine is more useful than vinblastine in lymphocytic leukemia. Another area of difference in clinical response to these drugs is acute leukemia, particularly in children; whereas vinblastine is rarely useful in this disease, vincristine is extremely effective.

The rapidity of action of vincristine and its lesser tendency for myelosuppressive action make it a more desirable agent for therapy in the presence of pancytopenia or in conjunction with other myelotoxic agents. It is particularly useful for the *induction* of remission in acute lymphoblastic leukemia in children when given with prednisone. It is the treatment of choice for this purpose and produces complete remissions in approximately 90% of children on the first course of antileukemic therapy (Bloomfield *et al.*, 1985). The approximate rate of second remissions is 70 to 80%. Vincristine and prednisone should be promptly discontinued after remission is induced, since other agents (*e.g.*, methotrexate and mercaptopurine) are more effective for *maintenance*. Vincristine has not prevented the occurrence of leukemia in the CNS. Beneficial responses have been reported in patients with a variety of other neoplasms, particularly Wilms' tumor, neuroblastoma, brain tumors, rhabdomyosarcoma, and carcinomas of the breast, bladder, and the male and female reproductive systems (Calabresi *et al.*, 1985).

The clinical toxicity of vincristine is mostly neurological, as described above. The more severe neurological manifestations may be avoided or reversed by either suspending therapy or reducing the dosage upon occurrence of the earliest symptoms, usually tingling and numbness of the extremities. Severe constipation, sometimes resulting in colicky abdominal pain and obstruction, may be prevented by a prophylactic program of laxatives and hydrophilic agents.

Alopecia occurs in about 20% of patients given vincristine; however, it is always reversible, frequently without cessation of therapy. Although less common than with vinblastine, leukopenia may occur with vincristine, and thrombocytopenia, anemia, polyuria, dysuria, fever, and gastrointestinal symptoms have been reported occasionally. Ischemic cardiac toxicity has been reported. The syndrome of hyponatremia associated with high urinary sodium and inappropriate ADH secretion has been occasionally observed during vincristine therapy. In view of the rapid action of the vinca alkaloids, it is advisable to take appropriate precautions to prevent the complication of hyperuricemia. This can be accomplished by the administration of *allopurinol* (*see* above).

VINDESINE

Preparation, Route of Administration, and Dosage. *Vindesine sulfate* (ELDISINE) is available in 5-mg vials. The drug is given intravenously with precautions to avoid extravasation. Various schedules are being tested, including slow infusion, single weekly injection, and multiple weekly injections. Single weekly doses of 3 to 4 mg/sq m are used in most schedules.

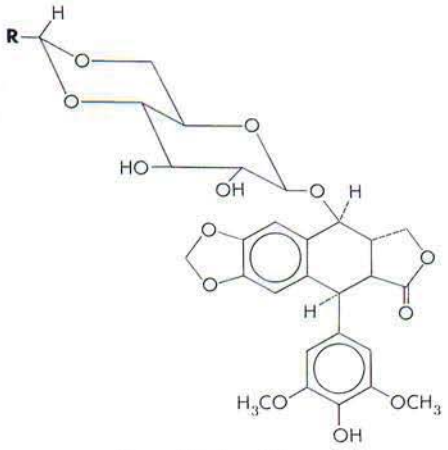
Therapeutic Uses and Clinical Toxicity. The clinical spectrum of activity of vindesine is still being evaluated. The drug appears effective in lymphomas, blastic crises of chronic granulocytic leukemia, and systemic mastocytosis. It is effective in neoplasms that are resistant to vincristine. The major toxic manifestations are moderate leukopenia and mild neurotoxicity.

EPIPODOPHYLLOTOXINS

Podophyllotoxin, extracted from the mandrake plant (or May apple), *Podophyllum peltatum*, was used as a folk remedy by the American Indians and early colonists for its emetic, cathartic, and anthelmintic effects. Two semisynthetic glycosides of the active principle, podophyllotoxin, have been developed that show significant therapeutic activity in several human neoplasms, including small-cell carcinomas of the lung, testicular tumors, Hodgkin's disease, and diffuse histiocytic lymphoma. These derivatives have been referred to as VP-16-213 (etoposide) and VM-26 (teniposide). Although podophyllotoxin binds to tubulin at a site distinct from that for interaction with the vinca alkaloids, etoposide and teniposide have no effect on microtubular structure or function at usual concentrations (Loike and Horwitz, 1976). (For reviews of the epipodophyllotoxins, *see* Bender and Chabner, 1982; Vogelzang *et al.*, 1982; D'Incalci and Garattini, 1983; O'Dwyer *et al.*, 1985.)

Chemistry. The chemical structures of etoposide and teniposide are shown below. They have been selected from many derivatives of

podophyllotoxin that have been synthesized during the past 20 years.



Etoposide: **R** = CH_3

Teniposide: **R** =

Mechanism of Action. Although the biochemical mechanisms of action are not yet understood, it appears that etoposide and teniposide are similar in their actions and in the spectrum of human tumors affected. Unlike podophyllotoxin, they do not cause mitotic arrest by binding to microtubules. Rather, at low concentrations, they block cells at the S–G_2 interface of the cell cycle and, at higher concentrations, cause G_2 arrest. Greatest lethality is seen in the S and G_2 phases. Single-strand DNA breaks are observed in intact cells but not with purified DNA, suggesting that cellular enzymes are in some way involved. Some evidence indicates that the epipodophyllotoxins stimulate DNA topoisomerase II to cleave DNA (Tewey *et al.*, 1984). It has also been reported that the epipodophyllotoxins, as well as the aglycone, can inhibit nucleoside transport and impair the incorporation of nucleosides into cellular nucleic acids.

ETOPOSIDE

Absorption, Fate, and Excretion. Oral administration of etoposide results in absorption of about 50% of the drug. After intravenous injection, peak plasma concentrations of 30 μg/ml are achieved; there is a biphasic pattern of clearance, with half-lives of about 3 hours and 12 hours. Approximately 45% of an administered dose is excreted in the urine, two thirds as the unchanged drug and one third as metabolites; 15% is recovered in the feces. Concentrations of etoposide in CSF range from 1 to 10% of the simultaneous value in plasma (Bender and Chabner, 1982; Wiemann and Calabresi, 1985).

Preparations, Dosage, and Routes of Administration. *Etoposide* (VEPESID) is available for intravenous administration. Investigational oral preparations are in the form of a drink ampul or a hydrophilic soft-gelatin capsule. The recommended intravenous dose is 50 to 100 mg/sq m, daily for 5 days, or 100 mg/sq m, on alternate days, for three doses. When given orally, the dose should be increased two times. Cycles of therapy are usually repeated every 3 to 4 weeks. The drug should be administered slowly during a 30- to 60-minute infusion in order to avoid hypotension and bronchospasm, probably due to the solvents used in the formulation.

Therapeutic Uses and Clinical Toxicity. Clinical use of etoposide has been primarily for testicular tumors that have not responded completely to vinblastine, bleomycin, and cisplatin. Although it is active alone, etoposide is frequently used in combination with cisplatin, bleomycin, and doxorubicin. The drug is active against small-cell and other carcinomas of the lung, Hodgkin's disease and non-Hodgkin's lymphomas, acute nonlymphocytic leukemia, carcinoma of the breast, and Kaposi's sarcoma associated with acquired immunodeficiency syndrome (AIDS). The dose-limiting toxicity of etoposide is leukopenia, with a nadir at 10 to 14 days and recovery by 3 weeks. Thrombocytopenia occurs less often and is usually not severe. Nausea, vomiting, stomatitis, and diarrhea occur in approximately 15% of patients treated intravenously, and in about 55% of patients who receive the drug orally. Alopecia is common but reversible. Fever, phlebitis, dermatitis, allergic reactions including anaphylaxis, and mild hepatic toxicity have been observed. Peripheral neuropathy is usually mild, but more severe if the drug is administered concomitantly with vincristine.

TENIPOSIDE

Teniposide is usually administered intravenously and has a multiphasic pattern of clearance from plasma. After distribution, half-lives of 4 hours and 10 to 40 hours are observed. Approximately 45% of the drug is excreted in the urine but, in contrast to etoposide, as much as 80% is recovered as metabolites. Less than 1% of the drug crosses the blood-brain barrier (Bender and Chabner, 1982; Wiemann and Calabresi, 1985).

Teniposide is available for investigational use. It is administered by intravenous infusion. The clinical spectrum of activity and the toxic manifestations of teniposide have not been fully determined but appear to be similar to those reported with etoposide.

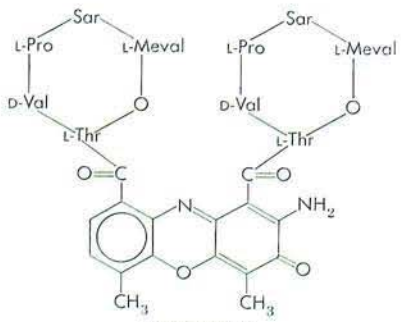
ANTIBIOTICS

DACTINOMYCIN (ACTINOMYCIN D)

History. The first crystalline antibiotic agent to be isolated from a culture broth of a species of *Streptomyces* was actinomycin A (Waksman and Woodruff, 1940). Many related antibiotics, including actinomycin D, have subsequently been obtained (Waksman Conference on Actinomycins, 1974). Dactinomycin has beneficial effects in the

treatment of a number of tumors, particularly certain neoplasms of childhood and choriocarcinoma.

Chemistry and Structure-Activity Relationship. The actinomycins are chromopeptides, and most of them contain the same chromophore, the planar phenoxazone *actinocin*, which is responsible for the yellow-red color of the compounds. The differences among naturally occurring actinomycins are confined to the peptide side chains, and the variations are in the structure, but not in the number or in the configuration of the α carbon, of the constituent amino acids. By varying the amino acid content of the growth medium it is possible to alter the types of actinomycins produced. Changes in the amino acid composition of both polypeptide chains can influence the biological activity of the molecule (Glaubiger and Ramu, 1982; Crooke, 1983). The chemical structure of dactinomycin is as follows:



Dactinomycin

(Sar = sarcosine
Meval = N-methylvaline)

Mechanism of Action. The capacity of actinomycins to bind with double-helical DNA is responsible for their biological activity and cytotoxicity. X-ray studies of a crystalline complex between dactinomycin and deoxyguanosine permitted formulation of a model that appears to explain the binding of the drug to DNA (Sobell, 1973). The planar phenoxazone ring intercalates between adjacent guanine-cytosine base pairs of DNA, where the guanine moieties are on opposite strands of the DNA. The summation of several interactions provides great stability to the dactinomycin-DNA complex, and, as a result of the binding of dactinomycin, the function of RNA polymerase and, thus, the transcription of the DNA molecule are blocked. The DNA-dependent RNA polymerases are much more sensitive to the effects of dactinomycin than are the DNA polymerases. (*See* Waksman Conference on Actinomycins, 1974; Goldberg *et al.*, 1977; Glaubiger and Ramu, 1982.)

Cytotoxic Action. The drug inhibits rapidly proliferating cells of normal and neoplastic origin and, on a molar basis, is among the most potent antitumor agents known. Atrophy of thymus, spleen, and other lymphatic tissues occurs in experimental animals. Detailed studies of the hematological, gastrointestinal, and other toxic effects of dactinomycin in animals have been described. It may produce damage to the hair roots and is capable of marked local inflammatory action. Erythema sometimes progressing to necrosis has been noted in areas of the skin exposed to x-radiation either before, during, or after administration of the drug.

Absorption, Fate, and Excretion. Dactinomycin is much less potent when given orally than when administered by parenteral injection. Very little active drug can be detected in the circulating blood 2 minutes after its intravenous injection. The drug is subsequently released from binding sites in tissues and disappears from plasma with a half-life of 36 hours. Metabolism of the drug is minimal. Dactinomycin does not cross the blood-brain barrier.

Preparation, Dosage, and Route of Administration. *Dactinomycin* (*actinomycin D;* COSMEGEN) is supplied as a lyophilized powder (0.5 mg in each vial). Solutions should not be exposed to direct sunlight. The usual daily dose is 10 to 15 μg/kg; this is given intravenously for 5 days; if no manifestations of toxicity are encountered, additional courses may be given at intervals of 3 to 4 weeks. Daily injections of 100 to 400 μg have been given to children for 10 to 14 days; in other regimens, 3 to 6 μg/kg, for a total of 125 μg/kg, and weekly maintenance doses of 7.5 μg/kg have been used. Although larger amounts have been given in more prolonged courses, in general the total dose necessary to produce antineoplastic effects has been approximately 2.5 to 5 mg. Although it is safer to administer the drug into the tubing of an intravenous infusion, direct intravenous injections have been given, with the precaution of discarding the needle used to withdraw the drug from the vial in order to avoid subcutaneous reaction.

Therapeutic Uses and Clinical Toxicity. The most important clinical use of dactinomycin is in the treatment of rhabdomyosarcoma and Wilms' tumor in children. In the latter case, remissions that last for several years and increased survival have been reported in patients with advanced disease, including pulmonary metastases (Waksman Conference on Actinomycins, 1974; Pinkel and Howarth, 1985). Antineoplastic activity has been noted in Ewing's tumor, Kaposi's sarcoma, and soft-tissue sarcomas. Its use together with vincristine and cyclophosphamide has been advocated in children with solid tumors. Dactinomycin can be effective in women with methotrexate-resistant choriocarcinoma. It may also be used with chlorambucil and methotrexate for patients with meta-

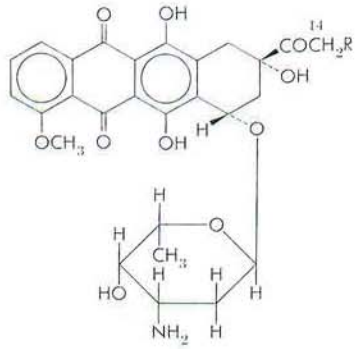
static testicular carcinomas, but this regimen is not preferable to the concurrent use of vinblastine, cisplatin, and bleomycin. It is of limited value in other neoplastic diseases of adults, although a response may sometimes be observed in patients with Hodgkin's disease and related lymphomas. Dactinomycin has also been used to inhibit immunological responses, particularly the rejection of renal transplants.

Toxic manifestations include anorexia, nausea, and vomiting, usually beginning a few hours after administration. Hematopoietic suppression with pancytopenia may occur from 1 to 7 days after completion of therapy. A decrease in the platelet count is often the first manifestation of bone-marrow depression, and pancytopenia may develop rapidly. Proctitis, diarrhea, glossitis, cheilitis, and ulcerations of the oral mucosa are common; dermatological manifestations include alopecia, as well as erythema, desquamation, and increased inflammation and pigmentation in areas previously or concomitantly subjected to x-radiation. Severe injury may occur as a result of local toxic action.

DAUNORUBICIN AND DOXORUBICIN

These anthracycline antibiotics and their derivatives are among the most important of the newer antitumor agents. They are produced by the fungus *Streptomyces peucetius* var. *caesius*. Daunorubicin was isolated independently by DiMarco and by Dubost and their colleagues in 1963. Doxorubicin was identified by Arcamone and coworkers in 1969. Although they differ only slightly in chemical structure, daunorubicin has been used primarily in the acute leukemias, whereas doxorubicin displays activity against a wide range of human neoplasms, including a variety of solid tumors. Unfortunately, the clinical value of both agents is limited by an unusual cardiomyopathy; its occurrence is related to the total dose of the drug, and it is often irreversible. In a search for agents with high antitumor activity but reduced cardiac toxicity, hundreds of anthracycline derivatives and related compounds have been prepared. Several of these have shown promise in the early stages of clinical study, including epirubicin and the synthetic compound mitoxantrone, which is an amino anthracenedione. (For reviews, *see* DiMarco, 1982; Myers, 1982, 1983; Gianni *et al.*, 1983; Myers *et al.*, 1984.)

Chemistry. The anthracycline antibiotics have tetracycline ring structures with an unusual sugar, daunosamine, attached by glycosidic linkage. Cytotoxic agents of this class all have quinone and hydroquinone moieties on adjacent rings that permit them to function as electron-accepting and -donating agents. Although there are marked differences in the clinical use of daunorubicin and doxorubicin, their chemical structures differ only by a single hydroxyl group on C 14. The chemical structures of daunorubicin and doxorubicin are as follows:



Daunorubicin: R = H
Doxorubicin: R = OH

Mechanism of Action. A number of important biochemical effects have been described for the anthracyclines and anthracenediones, any one or all of which could play a role in the therapeutic and toxic effects of such drugs. These compounds can intercalate with DNA. Many functions of DNA are affected, including DNA and RNA synthesis. Single- and double-strand breaks occur, as does sister chromatid exchange. Thus, the anthracyclines are both mutagenic and carcinogenic. Scission of DNA is perhaps related to the generation of free radicals. The anthracyclines react with microsomal cytochrome P-450 reductase in the presence of reduced nicotinamide adenine dinucleotide phosphate (NADPH) to form semiquinone radical intermediates, which, in turn, can react with oxygen to produce superoxide anion radicals. These can generate both hydrogen peroxide and hydroxyl radicals (·OH), which are highly destructive to cells. In addition, intramolecular electron-transfer reactions of the semiquinone intermediates result in the generation of other radicals and, thus, of potent alkylating agents. Furthermore, the anthracyclines can interact with cell membranes and alter their functions; there is evidence that this may play an important role in both the antitumor actions and the cardiac toxicity caused by anthracyclines (Tritton *et al.*, 1978).

As might be expected of compounds that inhibit DNA function, maximal toxicity occurs during the S phase of the cell cycle. At low concentrations of drug, cells will proceed through the S phase and die in G_2.

As discussed above, the phenomenon of pleiotropic drug resistance is observed with the anthracyclines. This appears to result from acceleration of the efflux of anthracyclines and other

agents from the cell. A membrane-associated glycoprotein, synthesized in high quantity as a result of gene amplification, has been implicated (Myers, 1982, 1983; Gianni *et al.*, 1983; Myers *et al.*, 1984).

Absorption, Fate, and Excretion. Daunorubicin and doxorubicin are usually administered intravenously, and they are then cleared from the plasma rapidly. The disappearance curve for doxorubicin is multiphasic, with elimination half-lives of 1.5 to 10 hours and 24 to 48 hours. There is rapid uptake of the drugs in the heart, kidneys, lungs, liver, and spleen. They do not appear to cross the blood-brain barrier.

There are notable differences in the metabolism of the two compounds. Daunorubicin is metabolized primarily to daunorubicinol. A significant fraction of doxorubicin is excreted unchanged, and there appear to be multiple metabolites, including, in particular, doxorubicinol. Aglycones result from further metabolism and, after conjugation, they are excreted in the bile. The hepatic clearance of doxorubicin has been estimated to be approximately 60% of hepatic blood flow, and severe clinical toxicity may result if the drug is administered to patients with impaired hepatic function. Renal excretion is modest and occurs mostly during the first 6 hours, resulting in a red discoloration of the urine. Modification of dosage is not required in patients with renal failure but is recommended when severe hepatic dysfunction and hyperbilirubinemia are present (Myers, 1982; Myers *et al.*, 1984; Wiemann and Calabresi, 1985).

Daunorubicin: Preparation, Dosage, and Route of Administration. *Daunorubicin (daunomycin, rubidomycin;* CERUBIDINE) is available as a lyophilized powder in 20-mg vials. The recommended dosage is 30 to 60 mg/sq m daily for 3 days or once weekly. The drug has also been given in doses of 0.8 to 1 mg/kg daily for 3 to 6 days, and other dosage schedules are being investigated. The agent is administered intravenously with appropriate care to prevent extravasation, since severe local vesicant action may result. Patients should be advised that the drug may impart a red color to the urine.

Daunorubicin: Therapeutic Uses and Clinical Toxicity. Daunorubicin is very useful in the treatment of acute lymphocytic and acute granulocytic leukemias. It is the single most active drug in acute nonlymphoblastic leukemia in adults and, given with cytarabine, is the treatment of choice in these conditions. The drug has some activity against solid tumors in children and in lymphomas; its activity against solid tumors in adults appears to be minimal.

The toxic manifestations of daunorubicin include bone-marrow depression, stomatitis, alopecia, gastrointestinal disturbances, and dermatological manifestations. Cardiac toxicity is a peculiar adverse effect observed with this agent. It is characterized by tachycardia, arrhythmias, dyspnea, hypotension, and congestive failure unresponsive to digitalis (*see* below).

Doxorubicin: Preparation, Dosage, and Route of Administration. *Doxorubicin hydrochloride* (ADRIAMYCIN) is supplied as a red-orange lyophilized powder in 10- and 50-mg vials. The recommended dose is 60 to 75 mg/sq m, administered as a single rapid intravenous infusion and repeated after 21 days. Care should be taken to avoid extravasation, since severe local vesicant action and tissue necrosis may result. Patients should be advised that the drug may impart a red color to the urine.

Doxorubicin: Therapeutic Uses and Clinical Toxicity. Doxorubicin is effective in acute leukemias and malignant lymphomas; however, in contrast to daunorubicin, it is also extremely active in a number of solid tumors. Used concurrently with cyclophosphamide, vincristine, bleomycin, and prednisone (BACOP), it is an important ingredient for the successful treatment of non-Hodgkin's lymphomas. In combination with bleomycin, vinblastine, and dacarbazine (ABVD), it is very effective in Hodgkin's disease. Together with cyclophosphamide and cisplatin, it has considerable activity against carcinoma of the ovary. It is a valuable component of various regimens of chemotherapy for carcinoma of the breast and small-cell carcinoma of the lung. The drug is also particularly beneficial in a wide range of sarcomas, including osteogenic, Ewing's, and soft-tissue sarcomas. It is one of the most active single agents for the treatment of metastatic adenocarcinoma of the breast, carcinoma of the bladder, bronchogenic carcinoma, and neuroblastoma. In metastatic thyroid carcinoma, doxorubicin is probably the best available agent. The drug has demonstrated activity in carcinomas of the endometrium, testes, prostate, cervix, and head and neck, and plasma-cell myeloma (DiMarco, 1975; Calabresi *et al.*, 1985).

The toxic manifestations of doxorubicin are similar to those of daunorubicin. Myelosuppression is a major dose-limiting complication, with leukopenia usually reaching a nadir during the second week of therapy and recovering by the fourth week; thrombocytopenia and anemia follow a similar pattern but are usually less pronounced. Stomatitis, gastrointestinal disturbances, and alopecia are common but reversible. Erythematous streaking near the site of infusion ("ADRIAMYCIN flare") is a benign local allergic reaction and should not be confused with extravasation. Facial flushing, conjunctivitis, and lacrimation may occur rarely. The drug may produce severe local toxicity in irradiated tissues (*e.g.*, the skin, heart, lung, esophagus, and gastro-

intestinal mucosa). Such reactions may occur even when the two therapies are not administered concomitantly.

Cardiomyopathy is a unique characteristic of the anthracycline antibiotics. Two types of cardiomyopathies may occur: (1) An acute form is characterized by abnormal ECG changes, including ST-T wave alterations and arrhythmias. This is brief and rarely a serious problem. Cineangiographic studies have shown an acute, reversible reduction in ejection fraction 24 hours after a single dose. An exaggerated manifestation of acute myocardial damage, the "pericarditis-myocarditis syndrome," may be characterized by severe disturbances in impulse conduction and frank congestive heart failure, often associated with pericardial effusion. (2) Chronic, cumulative dose-related toxicity is manifested by congestive heart failure that is unresponsive to digitalis. The mortality rate is in excess of 50%. Total dosage of doxorubicin as low as 250 mg/sq m can cause myocardial toxicity, as demonstrated by subendocardial biopsies. Nonspecific alterations, including a decrease in the number of myocardial fibrils, mitochondrial changes, and cellular degeneration, are visible by electron microscopy. The most promising noninvasive technic used to detect the early development of drug-induced congestive heart failure is radionuclide cineangiography. Although no completely practical and reliable predictive tests are available, the frequency of serious cardiomyopathy is negligible at total doses below 500 mg/sq m. The risk increases markedly (to >20% of patients) at total doses higher than 550 mg/sq m, and this total dosage should be exceeded only under exceptional circumstances. Cardiac irradiation or administration of cyclophosphamide or another anthracycline or related antibiotic increases the risk of cardiotoxicity. Because doxorubicin is primarily metabolized and excreted by the liver, it is important to reduce the dosage in patients with impaired hepatic function (Minow *et al.*, 1977; Bristow *et al.*, 1978; Myers, 1982; Wiemann and Calabresi, 1985).

BLEOMYCINS

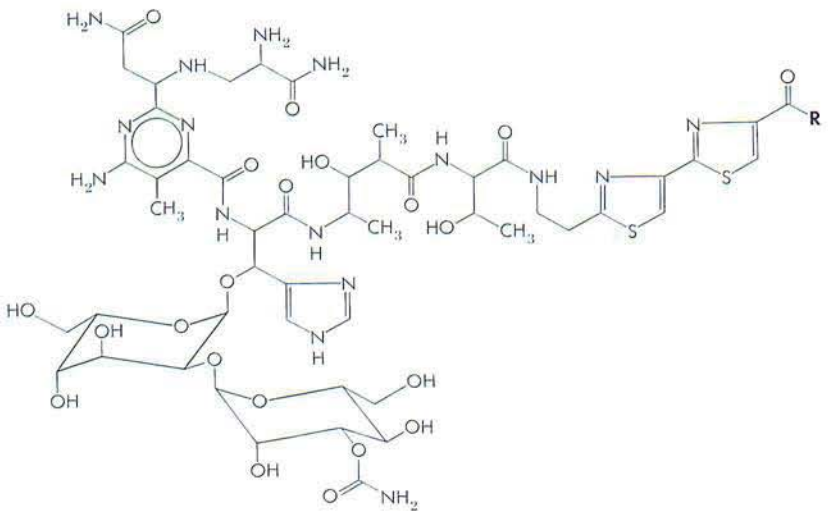
The bleomycins are an important group of antitumor agents discovered by Umezawa and colleagues as fermentation products of *Streptomyces verticillus*. The drug that is currently employed clinically is a mixture of copper-chelating glycopeptides that consists predominantly of two closely related agents, bleomycin A_2 and bleomycin B_2. The various bleomycins differ only in their terminal-amine moiety (*see* below), and the addition of various amines to fermentation broths have made possible the preparation of more than 200 different congeners. Evidence indicates that both the toxic effects and the antitumor spectrum can be modified by such changes.

Bleomycins have attracted great interest because of their activity in a variety of human tumors, including squamous carcinomas of skin, head, neck, and lungs, in addition to lymphomas and testicular tumors. In comparison with many other antineoplastic agents, the bleomycins in current use have minimal myelosuppressive and immunosuppressive activities. They do, however, cause unusual cutaneous and pulmonary toxicity. Since the toxic manifestations of the bleomycins do not overlap significantly with those of most other drugs and since their apparent mechanism of action is also unique (*see* below), the bleomycins have an important place in multidrug chemotherapy. (*See* Umezawa, 1973, 1979, 1982; Chabner, 1982a; Twentyman, 1984.)

Chemistry. The bleomycins are water-soluble, basic glycopeptides that differ from one another in their terminal-amine moieties. The structures of bleomycin A_2 and B_2 are shown on page 1286 (Oppenheimer *et al.*, 1979). The core of the bleomycin molecule is a complex structure containing a pyrimidine chromophore linked to propionamide, a β-aminoalanine amide side chain, and the sugars L-gulose and 3-O-carbamoyl-D-mannose. It also includes a side chain with the amino acids L-histidine and L-threonine, a methylvalerate residue, and a bithiazole carboxylic acid. The terminal amine is coupled through an amide linkage to this carboxylic acid. The bleomycins form equimolar complexes with cupric ions, with ligands involving the β-aminoalanine amide, the pyrimidine ring, the imidazole of L-histidine, and the carbamoyl group of mannose.

Mechanism of Action. While the bleomycins have a number of interesting biochemical properties, it seems most likely that their cytotoxic action relates to their ability to cause chain scission and fragmentation of DNA molecules. Studies *in vitro* indicate that bleomycin causes accumulation of cells in the G_2 phase of the cell cycle, and many of these cells display chromosomal aberrations, including chromatid breaks, gaps, and fragments as well as translocations.

Bleomycin appears to cause scission of DNA by interacting with O_2 and ferrous or cupric ions. Since Cu^{2+} binds to bleomycin more tightly than Fe^{2+}, it is likely that the cytotoxic agent is the copper-bleomycin complex (Lin *et al.*, 1983). In the presence of O_2 and a reducing agent, such as dithiothreitol, the metallobleomycin complex becomes activated and functions mechanistically as a mixed-function oxidase; that is, it resembles the actions of cytochrome P-450 (Ehrenfeld *et al.*, 1985). It has also been shown that metallobleomycin complexes can be activated by reaction with the flavin enzyme, NADPH–cytochrome P-450 reductase. Ble-



Bleomycinic Acid: **R** = OH

Bleomycin A_2: **R** = $NHCH_2CH_2CH_2-\overset{+}{S}(CH_3)-CH_3$

Bleomycin B_2: **R** = $NHCH_2CH_2CH_2CH_2NHC(=NH)NH_2$

omycin binds to DNA through intercalation. It is thought that the metallobleomycin complexes can generate free radicals by transferring electrons to molecular oxygen; the radicals so produced are presumed to be responsible for scission of the DNA chain. (*See* Sausville *et al.*, 1978a, 1978b; Povirk, 1979; Grollman and Takeshita, 1980; Chabner, 1982a.)

Of considerable interest is the apparent mechanism of the selective action of the bleomycins against squamous-cell carcinomas and their toxicity to lung and skin. Most tissues, except lung and skin, have relatively high activities of an enzyme, bleomycin hydrolase, that hydrolyzes the amide group of the β-aminoalanine amide of the bleomycin core and thereby inactivates the molecule. No correlation has been seen, however, between bleomycin hydrolase activity and sensitivity to bleomycin of tumor cells grown *in vitro*. This suggests that mechanisms of resistance other than increased drug degradation are operative (*see* Umezawa, 1979; Lazo *et al.*, 1982; Twentyman, 1984).

Absorption, Fate, and Excretion. Bleomycin is usually administered parenterally, and data on oral absorption are lacking. Relatively high concentrations of the drug are detected in the skin and lungs of experimental animals, the major sites of toxicity. Bleomycin does not cross the blood-brain barrier. In man, bleomycin localizes in various tumors, suggesting a lower level of inactivating enzyme at these sites.

After intravenous administration of a bolus dose of 15 units/sq m, peak concentrations of 1 to 10 mU/ml are achieved in plasma. The half-time for elimination is approximately 3 hours. After continuous intravenous infusion, the clearance of bleomycin is prolonged, with an elimination half-time of approximately 9 hours. The average steady-state concentration of bleomycin in plasma of patients receiving continuous intravenous infusions of 30 units daily for 4 to 5 days is approximately 150 mU/ml, and there is little bound to plasma proteins. Nearly two thirds of the drug is normally excreted in the urine, probably by glomerular filtration. Concentrations in plasma are greatly elevated if usual doses are given to patients with renal impairment. Doses of bleomycin should be reduced in the presence of severe renal failure (*see* Chabner, 1982a; Wiemann and Calabresi, 1985).

Preparation, Dosage, and Routes of Administration. *Bleomycin sulfate* (BLENOXANE) is available as a lyophilized powder in 15-unit vials to be reconstituted with sterile water, saline solution, or 5% dextrose solution. The recommended dose is 10 to 20 units/sq m, weekly or twice weekly, and the drug is most commonly administered intravenously or intramuscularly. It may also be given by

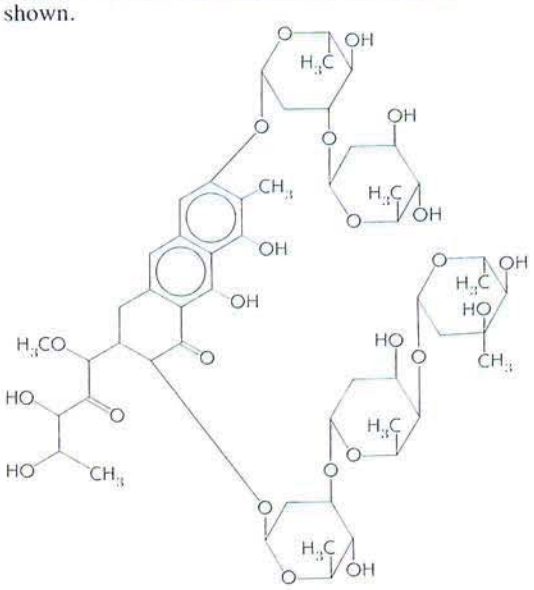
subcutaneous or intra-arterial injection. Total courses exceeding 400 units should be given with great caution because of a marked increase in the incidence of pulmonary toxicity; this may occur at lower doses when bleomycin is used concomitantly with other antineoplastic agents.

Therapeutic Uses and Clinical Toxicity. Bleomycin is effective in the treatment of testicular carcinomas. The overall response rate is approximately 30%, and this has increased to 90% when the drug is used with vinblastine. With the addition of cisplatin to this regimen, impressive numbers of complete remissions have been obtained that have lasted for several years (Williams and Einhorn, 1985). Bleomycin is also useful in the palliative treatment of squamous-cell carcinomas of the head, neck, esophagus, skin, and the genitourinary tract, including the cervix, vulva, scrotum, and penis. It is active in Hodgkin's disease and in other lymphomas (*see* Calabresi *et al.*, 1985).

In contrast to most other antineoplastic agents, bleomycin causes minimal bone-marrow toxicity. The most commonly encountered adverse effects are mucocutaneous reactions, including stomatitis and alopecia as well as hyperpigmentation, hyperkeratosis, pruritic erythema, ulceration, and vesiculation of the skin. These changes may begin with swelling and hyperesthesia of the hands or erythematous, ulcerating lesions over the pressure areas of the body. Recrudescence of mucocutaneous complications has been reported when other antineoplastic agents are used within 6 weeks after a course of bleomycin. The most serious adverse reaction to this drug is pulmonary toxicity. Injury to DNA and lipid peroxidation may be the initial lesions (Passero *et al.*, 1983). This poorly characterized manifestation may begin with decreasing pulmonary function, fine rales, cough, and diffuse basilar infiltrates, progressing to severe, and sometimes fatal, pulmonary fibrosis. Approximately 5 to 10% of patients receiving bleomycin develop this severe complication, and about 1% of all individuals treated with the drug have died of pulmonary toxicity. Pulmonary function studies have not been of predictive value. The risk is related to the total dose, with a significant increase in the incidence of pulmonary fibrosis noted at doses higher than 400 units and in patients over 70 years of age or with underlying pulmonary disease. The pulmonary toxicity of bleomycin may be potentiated by the administration of oxygen (Toledo *et al.*, 1982), by combination chemotherapy (Bauer *et al.*, 1983), and by previous radiation to the thorax. The use of corticosteroids has been advocated, but their value in reversing or preventing this complication remains to be established. Other toxic manifestations include hyperpyrexia, headache, nausea, and vomiting, as well as a peculiar, acute fulminant reaction observed in patients with lymphomas. This is characterized by profound hyperpyrexia, hypotension, and sustained cardiorespiratory collapse; it does not appear to be a classical anaphylactic reaction and may possibly be related to release of an endogenous pyrogen. Because this reaction has occurred in approximately 1% of patients with lymphomas and has resulted in deaths, it is recommended that patients with lymphomas receive a 1-unit test dose of bleomycin, followed by a 24-hour period of observation, before administration of the drug on standard dosage schedules. Unexplained exacerbations of rheumatoid arthritis have also been reported during bleomycin therapy. Raynaud's phenomenon and coronary artery disease have been reported in patients with testicular tumors treated with bleomycin in combination with other chemotherapeutic agents (Chabner, 1982a; Wiemann and Calabresi, 1985).

PLICAMYCIN (MITHRAMYCIN)

This cytotoxic antibiotic was isolated from cultures of *Streptomyces tanashiensis* by Rao and associates in 1962. Although the drug is highly toxic, it has some clinical value in the treatment of advanced embryonal tumors of the testes. Plicamycin appears to have a relatively specific effect on osteoclasts and lowers the plasma calcium concentrations in hypercalcemic patients, including those with various types of cancer and metastatic tumors in bone. The drug has been used experimentally in the treatment of symptomatic Paget's disease, and striking reductions in plasma alkaline phosphatase activity with concomitant relief of bone pain have been observed. For a discussion of the chemistry of plicamycin and related antibiotics, *see* Umezawa (1979). The structural formula of plicamycin is as shown.



Plicamycin

Mechanism of Action. Plicamycin intercalates into DNA in a manner similar to that of dactinomycin, with preferential binding to guanine-cytosine base pairs. In fact, these two drugs compete for the same binding sites on DNA. Inhibition of RNA, DNA, and protein synthesis is observed. However, these effects on macromolecular synthesis occur only at drug concentrations that are higher than those required to block tumor-cell growth. Thus, there is no established correlation between DNA binding and cytotoxicity.

The relatively specific effect of plicamycin on plasma concentrations of calcium suggests that the drug may have a direct action on bone (Robins and Jowsey, 1973). Studies with a tissue culture system of embryonic rat bone showed that the release of calcium caused by the addition of parathyroid hormone can be abolished by simultaneous treatment with low concentrations of plicamycin (Cortes *et al.*, 1972). These effects are thought to be the result of a direct action on osteoclasts (*see* Glaubiger and Ramu, 1982).

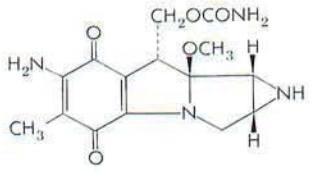
Absorption, Fate, and Excretion. Plicamycin is much less potent when administered orally than when given intravenously. Studies of its clinical pharmacology are lacking, and information on distribution, metabolic fate, and excretion is incomplete.

Preparation, Dosage, and Route of Administration. *Plicamycin (mithramycin;* MITHRACIN) is available as a freeze-dried powder in vials containing 2.5 mg of drug. The recommended dosage for treatment of testicular tumors is 25 to 30 μg/kg daily or on alternate days for eight to ten doses or until toxicity intervenes. The drug is usually diluted in 1 liter of 5% dextrose in water and administered by slow intravenous infusion over a period of 4 to 6 hours. Extravasation can cause local irritation and cellulitis. For the treatment of hypercalcemia or hypercalciuria, 25 μg/kg has been given daily for up to four doses; this is repeated at intervals of 1 week or more.

Therapeutic Uses and Clinical Toxicity. Plicamycin is of limited value in the treatment of neoplastic disease because of its severe toxicity. It has been beneficial in patients with disseminated testicular carcinomas, especially of the embryonal-cell type, but has been largely superseded by other drug regimens, particularly vinblastine, cisplatin, and bleomycin. The drug is useful in treating patients with severe hypercalcemia or hypercalciuria, particularly when associated with advanced or metastatic carcinoma that involves bone or produces parathyroid hormone–like substances. Its effectiveness in severe Paget's disease is encouraging but still considered investigational. Plicamycin is toxic to the bone marrow, liver, and kidneys. It produces a severe hemorrhagic diathesis, which may be the result of impaired synthesis of various clotting factors in addition to thrombocytopenia. Characteristically, this begins with epistaxis and may proceed to generalized hemorrhagic complications and even death. Adverse gastrointestinal, cutaneous, and neurological manifestations are also frequently observed. At the lower total dose recommended above for the treatment of hypercalcemia, toxicity is less severe.

MITOMYCIN

This antibiotic was isolated from *Streptomyces caespitosus* by Wakaki and associates in 1958. Mitomycin contains a urethane and a quinone group in its structure, as well as an aziridine ring, which is essential for antineoplastic activity. Of significance is that it acts through a bioreductive alkylation reaction and may be selectively toxic to hypoxic cells (Crooke and Bradner, 1976; Kennedy *et al.*, 1980; Glaubiger and Ramu, 1982). Its structural formula is as follows:



Mitomycin

Mechanism of Action. After intracellular enzymatic reduction of the quinone and loss of the methoxy group, mitomycin becomes a bifunctional or trifunctional alkylating agent. It inhibits DNA synthesis and cross-links DNA to an extent proportional to its content of guanine and cytosine. In addition, single-strand breakage of DNA is caused by reduced mitomycin; this can be prevented by free radical scavengers. Its action is most prominent during the late G_1 and early S phases of the cell cycle. Mitomycin is teratogenic and carcinogenic in rodents, but its immunosuppressive properties are relatively weak (Crooke and Bradner, 1976; Glaubiger and Ramu, 1982).

Absorption, Fate, and Excretion. Mitomycin is absorbed inconsistently from the gastrointestinal tract, and it is therefore administered intravenously. It disappears rapidly from the blood after injection. Peak concentrations in plasma are 1.5 μg/ml after doses of 20 mg/sq m. Mitomycin is cleared from plasma with a half-time of approximately 35 minutes (Reich, 1979). The drug is widely distributed throughout the body but is not detected in the brain. Inactivation occurs by metabolism, but the products have not been identified. It is metabolized primarily in the liver, and less than 10% of the active drug is excreted in the urine or the bile.

Preparation, Dosage, and Route of Administration. *Mitomycin (mitomycin C;* MUTAMYCIN) is available as deep blue-violet crystals in vials containing 5 or 20 mg. It is soluble in water and is readily reconstituted for administration through a running intravenous infusion. Extravasation may result in severe local injury. The currently recommended dosage is 2 mg/sq m daily for 5 days; this course is repeated after a 2-day interval. The same total dose (20 mg/sq m) may be administered intravenously as a single bolus infusion. The drug may be given again by these schedules after recovery from myelosuppressive toxicity.

Therapeutic Uses and Clinical Toxicity. Mitomycin is useful for the palliative treatment of gastric adenocarcinoma, in conjunction with fluorouracil and doxorubicin. It has produced temporary beneficial effects in carcinomas of the cervix, colon, rectum, pancreas, breast, bladder, head and neck.

and lung, and in melanoma. It has also shown activity against lymphomas and leukemia, particularly chronic granulocytic leukemia, but not in myeloma. All responses have been of brief duration and are complicated by severe toxicity. The major toxic effect is myelosuppression, characterized by marked leukopenia and thrombocytopenia; this may be delayed and cumulative. Nausea, vomiting, diarrhea, stomatitis, dermatitis, fever, and malaise have been observed. Interstitial pneumonia and glomerular damage resulting in renal failure are unusual but well-documented complications. Mitomycin may potentiate the cardiotoxicity of doxorubicin when used in conjunction with this drug (Wiemann and Calabresi, 1985).

ENZYMES

L-ASPARAGINASE

History. When L-asparaginase (L-asparagine amidohydrolase) was first introduced into cancer chemotherapy, it was believed that a distinct, qualitative biochemical difference had been detected between normal and certain malignant cells. Although this enzyme has found a limited place in the treatment of acute lymphoblastic leukemia, it is now appreciated that many normal tissues are also sensitive to L-asparaginase. Many toxic effects result from its impairment of the synthesis of secreted proteins, such as insulin, prothrombin and other clotting factors, albumin, and parathyroid hormone (Symposium, 1981a; Liu and Chabner, 1982; Wiemann and Calabresi, 1985).

Mechanism of Action. Most normal tissues synthesize L-asparagine in amounts sufficient for their metabolic needs. Certain neoplastic tissues, however, including acute lymphoblastic leukemic cells in children, require an exogenous source of this amino acid. L-Asparaginase, by catalyzing the hydrolysis of asparagine to aspartic acid and ammonia, deprives these malignant cells of the asparagine available from extracellular fluid, resulting in cellular death. There may be striking synergistic effects when asparaginase is used in combination with other drugs, such as methotrexate or cytarabine. The sequence of drug administration is crucial. For example, synergistic cytotoxicity is seen when methotrexate is administered before asparaginase. When the reverse sequence is used, the toxicity of methotrexate is attenuated. Several patients with refractory acute leukemias have responded favorably to such combinations. (For further discussion, *see* Capizzi and Handschumacher, 1982.)

Absorption, Fate, and Excretion. The enzyme is given parenterally. The rate of clearance from plasma varies considerably with different preparations; the half-life of EC-2 (*see* below) is from 11 to 23 hours (Broome, 1981; Liu and Chabner, 1982).

Preparation, Dosage, and Route of Administration. *Escherichia coli* produces two L-asparaginase isozymes, only one of which (EC-2) has antileukemic activity. The *E. coli* enzyme has been purified to homogeneity and is available for therapeutic use. *Asparaginase* (ELSPAR) is a dry powder in vials containing 10,000 international units (I.U.) per vial. The molecular weight of the enzyme is about 133,000, and it consists of four equivalent subunits (*see* Patterson, 1975). These preparations of *E. coli* L-asparaginase have weak glutaminase activity that may play a role in certain of the biological effects.

L-Asparaginase is administered intravenously or intramuscularly. The suggested dosage for the induction of remission in acute lymphoblastic leukemia is 200 I.U./kg daily for 28 days. Higher daily doses (1000 I.U./kg) for periods not exceeding 10 days have also been proposed as a method of avoiding anaphylaxis, which ordinarily appears only after the tenth day.

Therapeutic Uses and Clinical Toxicity. Unfortunately, L-asparaginase has not fulfilled its early promise of high tumoricidal activity with minimal toxicity in the treatment of human neoplasms. Complete remissions have been observed in acute lymphoblastic leukemia refractory to other antileukemic agents; the duration of these remissions, however, has been disappointingly short. Transient remissions have been observed in other forms of leukemia, and occasional beneficial responses have been reported in a few patients with malignant melanoma and T-cell lymphomas. Objective responses have not been seen with most solid tumors. The role of asparaginase in antineoplastic chemotherapy is currently limited to the treatment of acute lymphoblastic leukemia after standard regimens for induction of remission (*see* Symposium, 1981a).

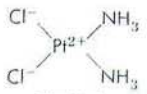
In contrast to most other antitumor drugs, L-asparaginase has minimal effects on the bone marrow, and it does not damage oral or intestinal mucosa or the hair follicles. On the other hand, severe toxicity has been observed that affects the liver, kidneys, pancreas, CNS, and the clotting mechanism. Biochemical evidence of hepatic dysfunction is present in more than 50% of those treated, and most patients display a substantial elevation of blood ammonia (as great as 700 to 900 μg/dl). Disorders of pancreatic function, including decreased insulin production, are often seen, and approximately 5% of treated adults develop overt pancreatitis; death has resulted from hemorrhagic pancreatitis. CNS dysfunction, ranging from depression to impaired sensorium and coma, has occurred in adults. It is suggested that all or most of these toxic effects result from inhibition of protein synthesis in various tissues of the body. L-Asparaginase has immunosuppressive activity, as seen by inhibition of antibody synthesis, delayed hypersensitivity, lymphocyte transformation, and graft rejection. Thus, both T- and B-lymphocyte functions are affected. Since L-asparaginase is a relatively large, foreign protein, it is antigenic, and hypersensitivity phenomena ranging from mild allergic reactions to anaphylactic shock have been reported in 5 to 20% of treated patients (Wiemann and Calabresi, 1985).

IV. Miscellaneous Agents

CISPLATIN

The platinum coordination complexes are cytotoxic agents that were first identified by Rosenberg and coworkers in 1965. Growth inhibition of *E. coli* was observed when electrical current was delivered between platinum electrodes. The inhibitory effects on bacterial replication were subsequently shown to be due to the formation of inorganic platinum-containing compounds in the presence of ammonium and chloride ions (Rosenberg *et al.*, 1965, 1967). *cis*-Diamminedichloroplatinum (II) (cisplatin) was found to be the most active of these substances in experimental tumor systems and has proven to be of clinical value (Rosenberg *et al.*, 1969; Rosenberg, 1973). Other platinum-containing compounds have subsequently been synthesized and tested; several new agents are currently in clinical trial. Despite pronounced nephrotoxicity and ototoxicity, cisplatin is very useful in combination chemotherapy of metastatic testicular and ovarian carcinoma; encouraging effects have also been reported during treatment of tumors of the bladder and of the head and neck (Rozencweig *et al.*, 1977; Connors, 1982; Zwelling and Kohn, 1982; Roberts, 1983; Hacker *et al.*, 1984; Symposium, 1984).

Chemistry. *cis*-Diamminedichloroplatinum (II) (cisplatin) is an inorganic water-soluble, platinum-containing complex. The *II* indicates the valence of platinum. The structural formula of cisplatin is relatively simple, as follows:



Cisplatin

The corresponding complex with the ammonia residues in the *trans* configuration lacks antitumor activity.

Mechanism of Action. Cisplatin appears to enter cells by diffusion. The chloride atoms may be displaced directly by reaction with nucleophils such as thiols; hydrolysis of chloride is probably responsible for formation of the activated species of the drug. The platinum complexes can react with DNA, forming both intrastrand and interstrand cross-links. The N(7) of guanine is very reactive, and cross-links between adjacent guanines on the same DNA strand are the most readily demonstrated. It is likely that the geometry of the *cis*, rather than the *trans*, form is more favorable for the formation of both intrastrand and interstrand cross-links. The formation of interstrand cross-links is a relatively slow process and occurs to a much smaller extent. The covalent binding of proteins to DNA has also been demonstrated. At present, there is no conclusive association between a single type of biochemical lesion and cytotoxicity. (*See* Zwelling and Kohn, 1982; Symposium, 1984.)

The specificity of cisplatin with regard to phase of the cell cycle appears to differ among cell types, although the effects on cross-linking are most pronounced during the S phase. Even though cisplatin is mutagenic, teratogenic, and carcinogenic, an increased incidence of second tumors, which has been observed with certain of the alkylating agents, has not yet been reported. Careful observations for a longer period of time are necessary before conclusions can be drawn on this important point.

In addition to its reactivity with DNA, cisplatin can react with other nucleophils, such as thiol groups of proteins. It is speculated that certain of the toxic effects of the drug, such as nephrotoxicity, ototoxicity, and intense emesis, may result from such reactions. This has led to the experimental testing of "rescue" technics that employ molecules with high affinity for heavy metals. One of these, diethyldithiolcarbamate (DDTC), a metabolite of disulfiram, has shown promise. When administered to animals 2 hours after treatment with cisplatin, renal and gastrointestinal toxicity is ameliorated, while the antileukemic effects are not prevented. This compound is under consideration for introduction into the clinic (Zwelling and Kohn, 1982; Borch *et al.*, 1984).

Cisplatin has immunosuppressive activity. Rejection of skin grafts and graft-versus-host responses are suppressed in animals, as is mitogenesis in lymphocytes stimulated by phytohemagglutinin (*see* Connors, 1982; Zwelling and Kohn, 1982; Roberts, 1983; Hacker *et al.*, 1984; Symposium, 1984).

Absorption, Fate, and Excretion. Cisplatin is not effective when administered orally. After rapid intravenous administration, the drug has an initial half-life in plasma of 25 to 50 minutes; concentrations decline subsequently with a half-life of 58 to 73 hours. More than 90% of the platinum in the blood is bound to plasma proteins. High concentrations of cisplatin are found in the kidney, liver, intestines, and testes, but there is poor penetration into the CNS. Only a small portion of the drug is excreted by the kidney during the first 6 hours; after 5 days up to 43% of the administered dose is recovered in the urine. When given by infusion instead of rapid injection, the plasma half-life is shorter and the amount of drug excreted is greater. The extent of bili-

ary or intestinal excretion of cisplatin is unknown (*see* Zwelling and Kohn, 1982; Wiemann and Calabresi, 1985).

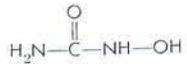
Preparation, Dosage, and Route of Administration. *Cisplatin* (PLATINOL) is available as a lyophilized powder in vials that contain 10 or 50 mg of drug. When used alone, the usual intravenous dose is 100 mg/sq m, given once every 4 weeks. Cisplatin is frequently used with other drugs in chemotherapy, and the dosage is reduced in such situations to 50 mg/sq m once every 3 weeks (when given with doxorubicin for ovarian neoplasms) or to 20 mg/sq m daily, for 5 consecutive days, every 3 weeks (when used in combination with bleomycin and vinblastine for testicular tumors). In order to prevent renal toxicity, hydration of the patient is recommended by the infusion of 1 to 2 liters of fluid for 8 to 12 hours prior to treatment. The appropriate amount of cisplatin is then diluted in a solution of dextrose, saline, and mannitol and administered intravenously over a period of 6 to 8 hours. Continued hydration to ensure adequate urinary output is recommended for 24 hours thereafter. Some investigators have advocated the concurrent administration of 40 mg of furosemide. Repeat courses of drug should not be given until all tests of renal and hematopoietic function, as well as auditory acuity, are within acceptable normal limits. Since aluminum reacts with and inactivates cisplatin, it is important not to use needles or other equipment that contain aluminum when preparing or administering the drug.

Therapeutic Uses and Clinical Toxicity. Cisplatin appears to be particularly effective in the treatment of testicular tumors when used alone or, preferably, with bleomycin and vinblastine (Williams and Einhorn, 1985). The drug is also beneficial in carcinoma of the ovary, particularly when used with doxorubicin (Durant and Omura, 1985). Cisplatin may also be useful in the treatment of carcinomas of the bladder, head and neck, and endometrium, as well as for chemotherapy of lymphomas and some neoplasms of childhood (*see* Rozencweig *et al.*, 1977; Sternberg *et al.*, 1977; Randolph *et al.*, 1978; Yagoda *et al.*, 1978; Einhorn and Williams, 1979).

The major toxicity caused by cisplatin is dose-related, cumulative impairment of renal tubular function; this usually occurs during the second week of therapy. When higher doses or repeated courses of the drug are given, irreversible renal damage may occur. Ototoxicity caused by cisplatin is manifested by tinnitus and hearing loss in the high-frequency range (4000 to 8000 Hz). It can be unilateral or bilateral, tends to be more frequent and severe with repeated doses, and may be more pronounced in children. Marked nausea and vomiting occur in almost all patients. Mild-to-moderate myelosuppression may occur with transient leukopenia and thrombocytopenia. Electrolyte disturbances, including hypomagnesemia, hypocalcemia, hypokalemia, and hypophosphatemia, have been encountered. Hypocalcemia and tetany secondary to hypomagnesemia have been observed, and routine measurement of magnesium concentrations in plasma is recommended. Hyperuricemia, peripheral neuropathies, seizures, and cardiac abnormalities have been reported. Anaphylactic-like reactions, characterized by facial edema, bronchoconstriction, tachycardia, and hypotension, may occur within minutes after administration and should be treated by intravenous injection of epinephrine and with corticosteroids or antihistamines (Wiemann and Calabresi, 1985).

HYDROXYUREA

First synthesized in 1869 by Dresler and Stein, hydroxyurea was found to produce leukopenia, anemia, and megaloblastic changes in the bone marrow of rabbits (Rosenthal *et al.*, 1928). It was later shown to have antineoplastic activity against sarcoma 180. Studies of its biological activity and assessments of clinical efficacy have been reviewed (Donehower, 1982). The structural formula of hydroxyurea is as follows:



Hydroxyurea

Cytotoxic Action. Hydroxyurea is representative of a group of compounds that have as their primary site of action the enzyme ribonucleoside diphosphate reductase. Other members of this class that have shown promise in the laboratory are guanazole and the α-N-heterocyclic carboxaldehyde thiosemicarbazones. A striking correlation has been observed between the relative growth rate of a series of rat hepatomas and the activity of ribonucleoside diphosphate reductase. This enzyme, which catalyzes the reductive conversion of ribonucleotides to deoxyribonucleotides, is a crucial and probably rate-limiting step in the biosynthesis of DNA, and it represents a logical target for the design of chemotherapeutic agents. Nonheme iron is an important component of this enzyme in mammalian tissues, and many of the active inhibitors can chelate or form complexes with iron. These compounds are specific for the S phase of the cell cycle and cause cells to arrest at the G_1–S interface. Since cells are highly sensitive to irradiation in the G_1 phase of the cycle, combinations of hydroxyurea and irradiation cause synergistic toxicity *in vitro* (Agrawal and Sartorelli, 1975; Donehower, 1982).

Two mechanisms of resistance to hydroxyurea have been proposed: the acquisition of ribonucleotide reductases with decreased sensitivity to hydroxyurea and marked increases in ribonucleotide reductase, perhaps due to gene amplification.

Absorption, Fate, and Excretion. In man, hydroxyurea is readily absorbed from the gastrointestinal tract, and peak plasma concentrations of 0.3 to 2.0 μM are reached in 1 to 2 hours; the plasma half-life is about 2 hours. Hydroxyurea readily crosses the blood-brain barrier. Approximately

80% of the drug is recovered in the urine within 12 hours after either oral or intravenous administration (Donehower, 1982).

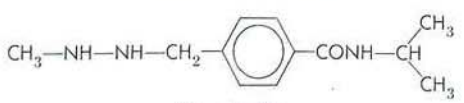
Preparation, Dosage, and Route of Administration. *Hydroxyurea* (HYDREA) is available for oral use in 500-mg capsules. Two dosage schedules are recommended: (1) intermittent therapy with 80 mg/kg, administered orally as a single dose every third day, and (2) continuous therapy with 20 to 30 mg/kg, administered orally as a single daily dose. Treatment should be continued for a period of 6 weeks in order to determine its effectiveness; if satisfactory antineoplastic results are obtained, therapy can be continued indefinitely, although leukocyte counts at weekly intervals are advisable.

Therapeutic Uses and Clinical Toxicity. At present, the primary role of hydroxyurea in chemotherapy appears to be in the management of selected myeloproliferative disorders, including chronic granulocytic leukemia, polycythemia vera, and essential thrombocytosis. It has also been effective in the hypereosinophilic syndrome (Parrillo *et al.*, 1978) and in achieving rapid reductions of markedly elevated blast cells in the peripheral blood of patients with acute granulocytic leukemia. Hydroxyurea has produced temporary remissions in patients with metastatic malignant melanoma and occasionally in those with other solid tumors, including carcinomas of the head and neck and genitourinary systems. Because of its ability to synchronize neoplastic cells *in vitro* in a radiation-sensitive phase of the cell cycle (G_1), it has been used in combination with radiotherapy in carcinomas of the cervix, head and neck, and lung.

Hematopoietic depression, involving leukopenia, megaloblastic anemia, and occasionally thrombocytopenia, is the major toxic effect; recovery of the bone marrow is usually prompt if the drug is discontinued for a few days. Other adverse reactions include gastrointestinal disturbances and mild dermatological reactions; more rarely, stomatitis, alopecia, and neurological manifestations have been encountered. Inflammation and increased pigmentation may occur in areas previously exposed to radiation.

PROCARBAZINE

A group of antitumor agents, the methylhydrazine derivatives, was discovered among a large number of substituted hydrazines, which had been originally synthesized as potential monoamine oxidase inhibitors. Antineoplastic effects in experimental tumors have been reported with several compounds in this series (Bollag, 1963), including procarbazine, an agent useful clinically in Hodgkin's disease. Comprehensive descriptions of the effects of procarbazine have been published (Oliverio, 1982; Wienkam *et al.*, 1982). The structural formula of procarbazine is as follows:



Procarbazine

Cytotoxic Action. Procarbazine itself is inert as a cytotoxic and mutagenic agent, and it must undergo metabolic activation to generate the proximal cytotoxic reactants. The activation pathways are complex and not yet fully understood. The first step involves oxidation of the hydrazine function with formation of the azo analog. This can occur spontaneously in neutral solution by reaction with molecular oxygen and can also occur enzymatically by reaction with the cytochrome P-450 system of the liver. Further oxidations can generate the methylazoxy and benzylazoxy intermediates. It is postulated that the methylazoxy compound can react further to liberate an entity resembling diazomethane, a potent methylating reagent. Free-radical intermediates may also be involved in cytotoxicity. Activated procarbazine can produce chromosomal damage, including chromatid breaks and translocations, that are consistent with its mutagenic and carcinogenic actions. Antimitotic effects have been described in a number of cell types; cells in the G_1 phase of the cell cycle are most susceptible. Inhibition of DNA, RNA, and protein synthesis has been detected both *in vitro* and *in vivo*. Although resistance to procarbazine develops rapidly, there is no clear notion of the mechanism. The highly lipophilic drug enters cells readily by diffusion (*see* Oliverio, 1982; Weinkam, *et al.*, 1982).

Absorption, Fate, and Excretion. Procarbazine is absorbed almost completely from the gastrointestinal tract. After parenteral administration, the drug is readily equilibrated between the plasma and the CSF. It is rapidly metabolized in man, and its half-life in the blood after intravenous injection is approximately 7 minutes. Oxidation of procarbazine produces the corresponding azo compound and hydrogen peroxide. Induction of microsomal enzymes by phenobarbital and other agents enhances the rate of conversion of procarbazine to its active metabolites; the potential for drug interaction thus exists when procarbazine is administered with other agents that are metabolized by microsomal enzymes. From 25 to 70% of an oral or parenteral dose given to man is recovered from the urine during the first 24 hours after administration; less than 5% is excreted as the unchanged compound, and the rest is mostly in the form of a metabolite, N-isopropylterephthalanic acid (Oliverio, 1973; Weinkam *et al.*, 1982).

Preparation, Dosage, and Route of Administration. *Procarbazine hydrochloride* (MATULANE) is marketed in 50-mg capsules. The recommended oral daily dose for adults is 2 to 4 mg/kg for the first week of therapy; then daily doses of 4 to 6 mg/kg are given until maximal response is obtained or toxicity intervenes. Daily maintenance doses of 1 to 2 mg/kg may be used.

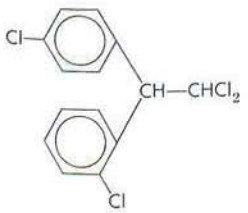
Therapeutic Uses and Clinical Toxicity. The greatest therapeutic effectiveness of procarbazine is in Hodgkin's disease, particularly when given with mechlorethamine, vincristine, and prednisone (the MOPP regimen) (DeVita and Hellman, 1982). Of major importance is the apparent lack of cross-

resistance with other antineoplastic agents. When used with various other agents, procarbazine has also demonstrated activity against small-cell carcinoma of the lung, non-Hodgkin's lymphomas, myeloma, melanoma, and brain tumors (Oliverio, 1973; Kreis, 1977; Weinkam *et al.*, 1982).

The most common toxic effects include leukopenia, thrombocytopenia, nausea, and vomiting, which occur in 50 to 90% of patients. Myelosuppression may begin during the second week of therapy, and its severity is dose dependent. Other gastrointestinal symptoms as well as neurological and dermatological manifestations have been noted in 5 to 10% of cases; psychic disturbances have also been reported. Because of augmentation of sedative effects, the concomitant use of CNS depressants should be avoided. The ingestion of alcohol by patients receiving procarbazine may cause intense warmth and reddening of the face, as well as other effects resembling the acetaldehyde syndrome produced by disulfiram. Since procarbazine is a weak monoamine oxidase inhibitor, hypertensive reactions may result from its use concurrently with sympathomimetic agents, tricyclic antidepressants, and foods with high tyramine content. Procarbazine is highly carcinogenic, mutagenic, and teratogenic. It is also a potent immunosuppressive agent.

MITOTANE (*o,p'*-DDD)

The principal application of mitotane, a compound chemically similar to the insecticides DDT and DDD, is in the treatment of neoplasms derived from the adrenal cortex. In studies of the toxicology of related insecticides in dogs, it was noted that the adrenal cortex was severely damaged, an effect caused by the presence of the *o,p'* isomer of DDD. Its structural formula is as follows:



Mitotane

Cytotoxic Action. The mechanism of action of mitotane has not been elucidated, but its relatively selective attack upon adrenocortical cells, normal or neoplastic, is well established. Thus, administration of the drug causes a rapid reduction in the levels of adrenocorticosteroids and their metabolites in blood and urine, a response that is useful both in guiding dosage and in following the course of hyperadrenocorticism (Cushing's syndrome) resulting from an adrenal tumor or hyperplasia. Damage to the liver, kidneys, or bone marrow has not been encountered.

Absorption, Fate, and Excretion. Clinical studies indicate that approximately 40% of the drug is absorbed after oral administration. After daily doses of 5 to 15 g, concentrations of 10 to 90 μg/ml of unchanged drug and 30 to 50 μg/ml of a metabolite are present in the blood. After discontinuation of therapy, plasma concentrations of mitotane are still measurable for 6 to 9 weeks. Although the drug is found in all tissues, fat is the primary site of storage. A water-soluble metabolite of mitotane is found in the urine; approximately 25% of an oral or parenteral dose is recovered in this form. About 60% of an oral dose is excreted unchanged in the stool.

Preparation, Dosage, and Route of Administration. *Mitotane* (*o,p'*-DDD; LYSODREN) is supplied in 500-mg scored tablets. Initial daily oral doses of 8 to 10 g are usually given in three or four divided portions, but the maximal tolerated dose may vary from 2 to 16 g per day. Treatment should be continued for at least 3 months; if beneficial effects are observed, therapy is maintained indefinitely. Spironolactone should not be administered concomitantly, since it interferes with the adrenal suppression produced by mitotane (Wortsman and Soler, 1977).

Therapeutic Uses and Clinical Toxicity. Mitotane is indicated in the palliative treatment of inoperable adrenocortical carcinoma. In addition to 138 patients reported by Hutter and Kayhoe (1966), 115 have been studied by Lubitz and associates (1973). Clinical effectiveness has been reported in 34 to 54% of these cases. Apparent cures have been reported in some patients with metastatic disease (Becker and Schumacher, 1975; Ostumi and Roginsky, 1975). Although the administration of mitotane produces anorexia and nausea in approximately 80% of patients, somnolence and lethargy in about 34%, and dermatitis in 15 to 20%, these effects do not contraindicate the use of the drug at lower doses. Since this drug damages the adrenal cortex, administration of adrenocorticosteroids is indicated, particularly in patients with evidence of adrenal insufficiency, shock, or severe trauma (Hogan *et al.*, 1978).

V. Hormones and Related Agents

ADRENOCORTICOSTEROIDS

The pharmacology, major therapeutic uses, and toxic effects of the adrenocorticosteroids are discussed in Chapter 63. Only the applications of the hormones in the treatment of neoplastic disease will be considered here. Because of their lympholytic effects and their ability to suppress mitosis in lymphocytes, the greatest value of these steroids is in the treatment of acute leukemia in children and of malignant lymphoma. They are especially effective in the management of frank hemolytic anemia and the hemorrhagic complications of thrombocytopenia that frequently accompany malignant lymphomas and chronic lymphocytic leukemia.

In acute lymphoblastic or undifferentiated leuke-

mia of childhood, adrenocorticosteroids may produce prompt clinical improvement and objective hematological remissions in 30 to 50% of children. Although these responses frequently are characterized by complete disappearance of all detectable leukemic cells from the peripheral blood and bone marrow, the duration of remission is extremely variable (2 weeks to 9 months) and relapse of the disease invariably occurs; eventually, drug resistance develops. Remissions occur more rapidly with corticosteroids than with antimetabolites, and there is no evidence of cross-resistance to unrelated agents. For these reasons, therapy is often initiated with a steroid and another type of agent, usually vincristine, in order to *induce* remissions. This approach, followed by continuous *maintenance* treatment with various agents, yields more prolonged remissions (*see* section on Methotrexate). Adult leukemia seldom responds to glucocorticoid therapy, but many symptoms of the disease, including the hemorrhagic manifestations of thrombocytopenia, may be controlled effectively, albeit temporarily, without demonstrable changes in platelet counts.

Corticosteroids have been useful in some patients with carcinoma of the breast and other carcinomas; however, palliative effects are of short duration and complications are frequent. Although the overall results in the treatment of carcinoma with these agents are disappointing, the judicious short-term use of corticosteroids may be indicated for specific complications such as hypercalcemia and intracranial metastases.

The adrenocorticosteroids are used in conjunction with x-ray therapy to reduce the occurrence of radiation edema in critical areas such as the superior mediastinum, brain, and spinal cord. These drugs are particularly useful in the symptomatic palliation of patients with severe hematopoietic depression secondary to bone-marrow involvement or previous radiation or chemotherapy. They may produce rapid symptomatic improvement in critically ill patients by temporarily suppressing fever, sweats, and pain, and by restoring, to some degree, appetite, lost weight, strength, and sense of well-being. The symptoms tend to recur after the hormone is withdrawn, which indicates that the effects of the disease, but not necessarily the disease process itself, have been affected. Therefore, the value of this type of therapy is to provide the patient with a relatively asymptomatic period during which the general physical condition may improve sufficiently to permit further definitive therapy.

Several preparations are available and at appropriate dosages exert similar effects (*see* Chapter 63). Prednisone, for example, is usually administered orally in doses as high as 60 to 100 mg, or even higher, for the first few days and gradually reduced to levels of 20 to 40 mg per day. A continuous attempt should be made to lower the dosage required to control the manifestations of the disease.

AMINOGLUTETHIMIDE

Originally developed as an anticonvulsant, aminoglutethimide was subsequently found to inhibit the synthesis of adrenocortical steroids (*see* Chapter 63). Aminoglutethimide inhibits the conversion of cholesterol to pregnenolone, the first step in the synthesis of cortisol. Inhibition of cortisol synthesis, however, results in a compensatory rise in the secretion of ACTH sufficient to overcome the adrenal blockade. Administration of dexamethasone does not prevent the increase in ACTH secretion because aminoglutethimide accelerates the metabolism of dexamethasone. Since the metabolism of hydrocortisone is not affected by aminoglutethimide, this combination produces reliable inhibition of the synthesis of cortisol (Santen *et al.*, 1980). Aminoglutethimide has been used to treat patients with adrenocortical carcinoma and Cushing's syndrome.

Although aminoglutethimide effectively blocks the secretion of cortisol, the production of other adrenal steroids, such as testosterone, dihydrotestosterone, androstenedione, progesterone, and 17-hydroxyprogesterone, is only partially inhibited. In certain tissues, including fat, muscle, and liver, androstenedione is converted by aromatization to estrone and estradiol. In postmenopausal and castrated women, the adrenal gland does not produce estrogens, but it is the most important source of precursors of estrogens. By inhibition of cytochrome P-450–dependent hydroxylation reactions that are necessary for aromatization reactions, aminoglutethimide is a potent inhibitor of the conversion of androgens to estrogens in extra-adrenal tissues. Patients treated with aminoglutethimide and hydrocortisone thus experience a lowering of plasma and urinary concentrations of estradiol that is equivalent to that observed in patients treated by surgical adrenalectomy (Santen *et al.*, 1982).

Therapeutic Uses and Clinical Toxicity. Aminoglutethimide is administered orally at a dose of 250 mg four times a day, together with 40 mg of hydrocortisone in divided doses. The largest dose of hydrocortisone, 20 mg, is given at night.

A major indication for the use of aminoglutethimide is to produce "medical adrenalectomy" in patients with advanced carcinoma of the breast, when the tumor contains estrogen receptors. If women are selected for therapy without regard to the status of estrogen receptors in the tumor, the response rate is 37%; patients whose tumor cells contain estrogen receptors experience a 50% response rate. Skin, soft tissue, and bone lesions respond more frequently than do other sites of metastasis. Such treatment is equal or superior to surgical adrenalectomy or hypophysectomy (Harvey *et al.*, 1979).

Early toxic effects of aminoglutethimide include lethargy, visual blurring, drowsiness, and ataxia. These symptoms usually resolve after 4 to 6 weeks of treatment. A pruritic, maculopapular rash usually appears 10 days after treatment is initiated and resolves after approximately 5 days without withdrawal of the drug. Since the adrenal recovers normal secretory activity and the response to stress 36 hours after aminoglutethimide and hydrocortisone are withdrawn, it is not necessary to taper the administration of these drugs.

PROGESTINS

Progestational agents (*see* Chapter 61) have been found useful in the management of patients with endometrial carcinoma previously treated by surgery and radiotherapy. These compounds were tried initially because of the concept that carcinoma of the endometrium results from the prolonged, unopposed overstimulation by estrogen. This led to the use of progesterone, which would correct this situation because of its physiological effect in producing maturation and secretory activity of the normal endometrium. Apparently a portion of neoplastic cells arising from this tissue is still influenced by normal hormonal controls.

There are several preparations available. Hydroxyprogesterone caproate is usually administered intramuscularly in doses of 500 mg twice weekly; medroxyprogesterone acetate can be administered intramuscularly in doses of 400 mg twice weekly. An alternative oral agent is megestrol acetate (40 to 320 mg daily, in divided doses). Beneficial effects, usually characterized by regression of pulmonary metastases, have been observed in approximately one third of patients. Responses to progestational agents have also been reported in metastatic carcinomas of the breast and prostate, and in hypernephromas.

ESTROGENS AND ANDROGENS

A discussion of the pharmacology of the estrogens and androgens appears in Chapters 61 and 62. Their use in the treatment of certain neoplastic diseases will be discussed here. They are of value in this connection because certain organs that are often the primary site of growth, notably the prostate and the mammary gland, are dependent upon hormones for their growth, function, and morphological integrity. Carcinomas arising from these organs often retain some of the hormonal requirements of their normal counterparts for varying periods of time. By changing the hormonal environment of such tumors it is possible to alter the course of the neoplastic process.

Androgen-Control Therapy of Prostatic Carcinoma. The development of the androgen-control regimen for the treatment of prostatic carcinoma is largely the contribution of Huggins and associates (1941). Although no case of prostatic carcinoma has been cured by androgen-control therapy, life expectancy has been increased and thousands of patients have enjoyed the benefit of its ameliorating effects. Approximately 95% of patients with clinical manifestations of carcinoma of the prostate have nonresectable disease and require androgen-control therapy.

History and Rationale. The relationship between the prostate and testicular function was appreciated early in the nineteenth century, when it was noted that regression of the prostate followed orchiectomy. Huggins observed that, in the dog, shrinkage of the gland and cessation of secretion followed castration and that these effects could be reversed by the administration of androgen. Of even greater significance was the observation that the administration of estrogen could block the effects of the androgen. On the basis of these experimental findings, Huggins and associates (1941) postulated that significant clinical improvement should occur after bilateral orchiectomy in patients with advanced prostatic carcinoma, a theory that proved to be correct. It was also demonstrated that similar results could be obtained by the administration of estrogen (Herbst, 1941). The fundamental mechanism by which the lack of androgen results in regressive changes in normal and neoplastic prostatic cells is unknown. Unfortunately, relapse eventually occurs in patients on androgen-control therapy.

Therapeutic Regimen. Control of prostatic cancer is most effectively obtained by the combined use of orchiectomy and estrogen in patients who, when first treated, are free from metastases. When metastases are already present, orchiectomy seems to be more effective than estrogen therapy, and their combination does not appear to offer any advantage. When either orchiectomy or estrogen alone is employed as a therapeutic measure and the patient relapses, some degree of symptomatic improvement may be obtained by the alternative procedure.

The choice of estrogen is largely determined by cost and convenience. Diethylstilbestrol or a related synthetic compound is usually the preparation of choice. There is no evidence that survival is improved with excessively large doses. An average dose of diethylstilbestrol is 5 mg three times daily. Indeed, many oncologists reduce the daily dose to as little as 1 mg after a few weeks. The dose of other estrogens is in proportion to their potency.

Response to Therapy. Subjective and objective improvements rapidly follow the institution of androgen-control therapy of prostatic carcinoma. From the patient's point of view the most gratifying of these is relief of pain. This is associated with an increase in appetite, weight gain, and a feeling of well-being. Objectively, there are regressions of the primary tumor and soft-tissue metastases, but neoplastic cells do not disappear completely. Elevated plasma acid phosphatase activity usually returns to normal. Alkaline phosphatase activity may first rise and then fall. There is often an associated recovery from anemia. Some patients with prostatic carcinoma show no response to androgen-control therapy. Eventually prostatic tumors become insensitive to the lack of androgen or the presence of estrogen; however, it is now well established that effective palliation is afforded by the therapeutic regimen and that the life expectancy of the treated patient is significantly increased.

Androgen-Control Therapy of Carcinoma of the Male Breast. Carcinoma of the male breast is a rare tumor that is seldom diagnosed sufficiently early to permit definitive surgical intervention. The neoplasm regresses in a high proportion of cases in response to androgen-control therapy. Although this may be achieved by either orchiectomy or the administration of estrogen, it is preferable to initiate treatment with orchiectomy; when evidence of exacerbation appears, estrogen therapy is insti-

tuted. Remissions of several years can be achieved with this therapeutic regimen.

Untoward Effects of Androgen-Control Therapy. Androgen-control therapy is one of the safest forms of cancer chemotherapy. The psychic trauma of orchiectomy is not inconsequential, but is often tempered by the age of the patient. After orchiectomy alone, hot flushes are not uncommon; these can be controlled by the administration of estrogen. Estrogens are capable of producing the untoward responses described in detail in Chapter 61. Mild gastrointestinal disturbances may be noted; occasionally, these may be severe enough to require discontinuation of the drug. There may be some expansion of extracellular fluid volume in patients with poor cardiac function. There is also significant mortality from cardiac and cerebrovascular complications. Gynecomastia is frequent and may be a disturbing feature in some patients. In rare instances, carcinoma of the male breast has occurred in patients given estrogen for prolonged periods of time.

Estrogens and Androgens in the Treatment of Mammary Carcinoma. Estrogens and androgens have found application in the treatment of advanced mammary carcinoma. The hormones afford some measure of relief in patients with nonresectable disease in whom the metastatic lesions are too widespread to permit effective radiation.

Therapeutic Regimen. The therapeutic regimen for the use of androgens and estrogens in the treatment of carcinoma of the breast is largely empirical. The first cardinal principle is that hormonal therapy should be reserved for patients for whom surgical treatment or radiotherapy has been fully considered and deemed no longer of value. Once this qualification has been met, androgen therapy may be employed for patients in any age group. Objective remissions are obtained in approximately 20% of patients. Estrogen therapy generally is contraindicated in patients who are not at least 5 years past the menopause, regardless of chronological age. Experience has shown that estrogen may accelerate the neoplastic process in women who are still menstruating. In premenopausal women, oophorectomy is the first recommended procedure to institute hormonal control. On the basis of earlier observations, androgen was said to be preferable for the treatment of bone metastases, whereas estrogen was considered to be the preparation of choice for soft-tissue metastases. Subsequent evidence does not entirely substantiate these findings, however, and it is often the practice to change from one type of hormone to the other in unresponsive patients.

Progress in endocrinology has led to the development of methods that are very useful for the selection of patients for ablative or additive hormonal therapy. Tissues that are responsive to estrogens contain receptors for the hormones that can be detected by ligand-binding technics. Carcinomas that lack specific estrogen-binding capacity rarely respond to hormonal manipulation. The tumors that contain receptors usually do respond and, furthermore, are associated with a better overall prognosis independent of the type of therapy.

Hormonal therapy utilizes doses much larger than those needed for physiological replacement. Androgen therapy with oral agents is preferable; a common regimen is fluoxymesterone, 10 mg orally three times a day. Parenteral androgen therapy may be given as dromostanolone propionate, 100 mg intramuscularly three times weekly.

Compounds with estrogenic activity are numerous. Oral diethylstilbestrol is the most frequently used; it is given initially in doses of 5 mg daily. This dose is gradually increased to a maintenance dose of 5 mg three times daily over a 1- to 2-week period. Ethinyl estradiol is also commonly used, the dosage being gradually increased from 0.5 mg orally once daily to the customary maintenance dose of 3 mg daily, given in three portions. Ethinyl estradiol may be tried if diethylstilbestrol causes intolerable gastrointestinal side effects.

Response to Therapy. The onset of action of the hormones is slow, and it is necessary to continue therapy for 8 to 12 weeks before a decision can be reached as to effectiveness. If a favorable response is obtained, hormonal treatment should be continued until an exacerbation of symptoms occurs. Withdrawal of the hormone at this time may occasionally be followed by another remission. The duration of an induced remission averages about 6 months to 1 year; however, some patients may receive benefit for several years.

Untoward Effects. All the untoward effects that commonly accompany estrogen and androgen therapy have been observed in the use of these agents in the treatment of mammary carcinoma; these effects are described in Chapters 61 and 62. Two toxic manifestations require emphasis. With either hormone, the combined effect of a steroid and osteolytic metastases may result in marked hypercalcemia. The chief dangers are ectopic calcification, particularly in the urinary tract, and the physiological disturbances that may accompany an increase in the concentration of ionized calcium in the extracellular fluid. Patients who show an elevation in plasma calcium should receive a high fluid intake. Severe hypercalcemia, whether spontaneous or drug induced, is a true medical emergency. If an estrogen or androgen is being used, it should be discontinued. Forced hydration, by vein if the patient cannot drink, is mandatory. Further measures may be necessary; these include administration of diuretics, adrenocorticosteroids in large doses, oral or intravenous phosphate supplementation, or the intravenous administration of plicamycin (*see* above; *see also* Chapter 65). When drug-induced hypercalcemia is corrected, further therapy may be cautiously attempted. The incidence of hypercalcemia in patients receiving androgens is approximately 10%; it occurs less frequently with estrogen therapy. Plasma calcium concentrations should be determined routinely in patients receiving hormonal therapy.

Rarely, either estrogen or androgen therapy may cause exacerbation of the neoplastic process; this occurs more frequently as a result of estrogen administration.

ANTIESTROGENS

TAMOXIFEN

About one third of patients with advanced carcinoma of the breast benefit from either endocrine ablation or hormonal therapy. The growth of certain breast cancer cells depends on the presence of estrogens and, in these cases, oophorectomy may suppress tumor growth. A new development has been the introduction of effective and relatively nontoxic antiestrogenic agents that block the peripheral functions of estrogens on target tissues (*see* Chapter 61). Of various compounds tested, *tamoxifen* has been approved for clinical use in the United States; it is effective, palliative treatment for certain patients with advanced breast cancer. Tumors that contain estrogen receptors and those whose growth was slowed by prior hormonal therapy tend to respond to tamoxifen; others are often insensitive (*see* Tormey *et al.*, 1976; Kiang and Kennedy, 1977; Moseson *et al.*, 1978; Jordan, 1982a). The structural formula of tamoxifen is shown in Chapter 61.

Mechanism of Action. Estrogen receptors can be detected in tumor cells of 50% of premenopausal women with breast cancer and 75% of postmenopausal women. Antiestrogens, such as tamoxifen, bind to estrogen receptors in a fashion similar to that of estradiol. The complex of the receptor and the antiestrogen may bind to nuclear chromatin in an atypical manner and for a longer time than the normal hormone-receptor complex. Furthermore, antiestrogens may deplete the cytoplasm of free receptor. Either or both of these effects could severely impair the continued growth of an estrogen-dependent tumor. These observations offer a sound rationale for the use of antiestrogen therapy in combination with various ablative operations—oophorectomy, adrenalectomy, or hypophysectomy. Although any of these procedures may decrease the concentrations of estrogens in tissues, they do not completely eliminate the synthesis of the hormones. For example, after oophorectomy, androgens produced by the adrenals may be converted to estradiol in peripheral tissues. Three antiestrogens have shown useful actions in the treatment of human breast cancer: *clomiphene*, *nafoxidine*, and *tamoxifen*. Of these, tamoxifen is favored because of its relative lack of toxicity (*see* Legha *et al.*, 1978).

Absorption, Fate, and Excretion. After oral administration, peak concentrations of tamoxifen are found in blood after 4 to 7 hours. The decline in plasma concentration is biphasic; the initial $t_{1/2}$ is 7 to 14 hours, and the terminal $t_{1/2}$ is longer than 7 days. Repeated administration of tamoxifen results in accumulation of the drug, and steady-state concentrations are achieved in 4 weeks. The principal metabolite of tamoxifen in man is N-desmethyltamoxifen. Concentrations of this metabolite in blood are approximately twice those of the parent compound at steady state. Studies in animals indicate that tamoxifen undergoes extensive metabolic conversion by hydroxylation and conjugation. The monohydroxylated derivative has more antiestrogenic activity than does the parent compound or the dihydroxylated metabolite. After enterohepatic circulation, glucuronides and other metabolites are excreted in the stool; excretion in the urine is minimal (Jordan, 1982a).

Preparation, Dosage, and Route of Administration. *Tamoxifen citrate* (NOLVADEX) is marketed in 10-mg tablets. The recommended dose is 20 to 40 mg daily, administered orally in two divided doses. Objective responses usually occur in 4 to 10 weeks but may be delayed for several months in patients with bone metastases.

Therapeutic Uses and Clinical Toxicity. Tamoxifen is useful in the palliative treatment of advanced carcinoma of the breast in postmenopausal women. Patients who have tumors that contain estrogen receptors are most likely to respond to the drug; those with a recent negative assay for receptor-binding activity are unlikely to benefit. Although a few premenopausal women have responded to this agent, it is more effective in patients who are several years postmenopausal, have metastases to soft tissues rather than to bone, and have derived beneficial effects from previous hormone therapy.

The most frequent adverse reactions include hot flashes, nausea, and vomiting. These may occur in approximately 25% of patients and are rarely severe enough to necessitate discontinuation of therapy. Menstrual irregularities, vaginal bleeding and discharge, pruritus vulvae, and dermatitis have occurred less frequently. The occurrence of pain in tumors, particularly bone metastases, as well as local flare of disease, characterized by increase in size and marked erythema of the lesions, is sometimes associated with good responses. Other infrequent adverse effects include hypercalcemia, peripheral edema, anorexia, depression, pulmonary embolism, light-headedness, headache, mild-to-moderate thrombocytopenia, and leukopenia. Tamoxifen is said to be carcinogenic and teratogenic in animals. (Further information about tamoxifen may be found in the publications of Lerner *et al.*, 1976; Manni *et al.*, 1976; Tormey *et al.*, 1976; Kiang and Kennedy, 1977; Young *et al.*, 1977; Kiang *et al.*, 1978; Legha *et al.*, 1978; Moseson *et al.*, 1978; Furr and Jordan, 1984.)

GONADOTROPIN-RELEASING HORMONE ANALOGS

A recent development of considerable interest is the synthesis of various analogs of gonadotropin-releasing hormone (*see* Chapter 59). Marked decrease in circulating concentrations of gonadotropins and testosterone can be induced in patients with prostatic carcinoma treated with *leuprolide*, a peptide analog with high agonist activity. This compound has paradoxical effects on the pituitary; it stimulates initially, but there is subsequent inhibition of secretion of follicle-stimulating hormone

(FSH) and luteinizing hormone (LH). Impressive beneficial effects have been obtained in initial clinical studies, and large-scale clinical trials with leuprolide are in progress. The incidence of side effects, including gynecomastia, edema, thromboembolism, and nausea and vomiting, may be significantly lower in patients treated with leuprolide compared with those receiving therapeutically equivalent doses of diethylstilbestrol (Leuprolide Study Group, 1984; Torti, 1984).

VI. Immunosuppressive Agents

While many agents with immunosuppressive activity are discussed throughout this chapter, cyclosporine is unique in its structure and its mechanism of action. Of great importance, it does not share the relatively general cytotoxic activity of other drugs used for this purpose.

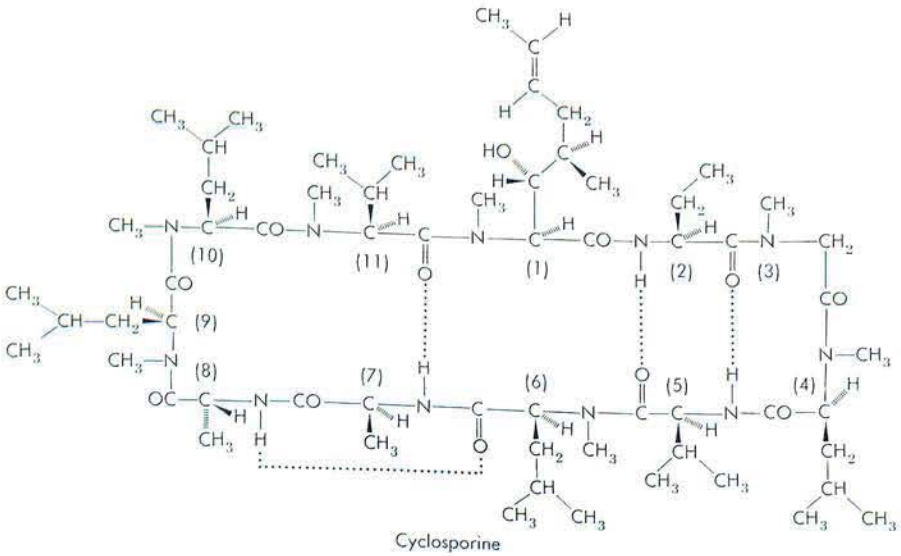
CYCLOSPORINE

Cyclosporine (formerly called cyclosporin A) is a hydrophobic cyclic undecapeptide produced by the fungus *Tolypocladium inflatum*. It is an immunomodulatory substance that acts specifically at an early stage in the activation of T lymphocytes. It displays little myelotoxicity and does not adversely affect the phagocytic cells of the reticuloendothelial system. Thus, in contrast to azathioprine and other cytotoxic agents, cyclosporine makes possible the suppression of T-cell–mediated cellular immunity without causing major effects on the antibacterial defenses of the body. Its introduction into the clinic in recent years has played a major role in suppression of the immunological reactions that have adversely affected the fields of organ and bone-marrow transplantation in the past. The use of cyclosporine has also been promising in animal models of autoimmune disease, and clinical studies of such therapy have been initiated. (For a comprehensive discussion, *see* Symposium, 1983b.)

Chemistry. Cyclosporine is a complex cyclic peptide. It is comprised of 11 amino acid residues, and it is remarkably hydrophobic. All of the amino acids have the L configuration, except for D-alanine in position 8 and sarcosine (N-methylglycine) in position 3. Seven of the amino acids are N-methylated. The four remaining protonated peptide nitrogen atoms can form hydrogen bonds with carbonyl groups, which contribute substantially to the rigidity of the cyclosporine skeleton. A unique feature of the molecule is the nine-carbon olefin-containing amino acid in position 1. The structure of cyclosporine is shown below.

The total synthesis of cyclosporine has been accomplished, and a number of modified derivatives have been produced. Results to date indicate that the unusual nine-carbon amino acid is intimately involved in the biological actions of the molecule (*see* Wenger, 1983).

Mechanism of Action. Although the precise biochemical events are not yet understood, evidence indicates that cyclosporine blocks an early stage in the activation of



Cyclosporine

cytotoxic T lymphocytes in response to alloantigens. Several important effects have been observed. Production of the soluble proliferative factor, interleukin II, by activated T-helper cells is blocked; the acquisition of receptors for interleukin II by precursor cytotoxic T cells is inhibited; and the responsiveness of helper/inducer T cells to interleukin I is diminished. Cyclosporine has little effect, however, on the activation and proliferation of suppressor T lymphocytes or on the responsiveness to interleukin II of primed T lymphocytes. Other potentially important effects are inhibition of the production of interferon by lymphocytes and impaired production of macrophage-specific lymphokines. (For further discussion, *see* Hess *et al.*, 1983; Kahan *et al.*, 1983; Lafferty *et al.*, 1983.)

Specific cytosolic cyclosporine-binding proteins have been discovered in lymphoid tissues. Two closely related proteins have been identified and purified to homogeneity from bovine thymocytes. These basic proteins, referred to as cyclophilins, have molecular weights of about 15,000. Cyclophilins with similar molecular weights but different isoelectric points have been found in cytosolic fractions from murine and human thymus and mature T cells. Cyclophilin has also been identified in nonlymphoid tissues; concentrations are high in brain and kidney, organs that display toxic effects during treatment with cyclosporine. It seems likely that cyclophilin plays a crucial role in the activation of T lymphocytes. Its presence in other tissues presumably indicates that it has other important biological functions (Handschumacher *et al.*, 1984).

Absorption, Fate, and Excretion. After oral administration, absorption of cyclosporine is variable and incomplete, with an absolute bioavailability of approximately 20 to 50%. Peak concentrations in plasma and blood are achieved after approximately 2 to 4 hours; these values are about 1 ng/ml for plasma and 1.4 to 2.7 ng/ml for blood for each milligram of drug administered. About 50% of the drug is found in erythrocytes, 40% in plasma, and 10% in leukocytes. In plasma, approximately 95% is bound to proteins, mostly lipoproteins. Although no major metabolic pathway has been identified, the drug is almost completely metabolized, and less than 0.1% of intact compound is recovered in the urine. Many metabolites are excreted in the bile. A biphasic disappearance curve from blood has been observed, with a terminal half-life of 10 to 27 hours (Kahan *et al.*, 1983; Weil, 1984).

Preparations, Routes of Administration, and Dosage. *Cyclosporine* (SANDIMMUNE) is marketed as an immunosuppressive agent for the prophylaxis and treatment of the rejection of transplanted organs. It is provided as an oral solution of 100 mg/ml with 12.5% alcohol and for intravenous administration as a solution of 50 mg/ml, with 33% alcohol and 650 mg of polyoxyethylated castor oil. The usual oral dose is 10 to 15 mg/kg daily, starting a few hours before transplantation and continuing for 1 to 2 weeks; dosage is then reduced gradually to a maintenance level of 5 to 10 mg/kg daily. The drug may be administered intravenously, as a dilute solution of 50 mg per 20 to 100 ml of normal saline solution or 5% dextrose in water, by slow infusion over a period of 2 to 6 hours. The intravenous dose is approximately one third the oral dose.

Therapeutic Use and Clinical Toxicity. Cyclosporine is a potent immunosuppressive agent and is used for prophylaxis and treatment of organ rejection in allogeneic transplants, usually in conjunction with adrenocorticosteroids. Prolonged survival of allogeneic transplants of the kidney, liver, and heart has been documented in man. There has been more limited, but successful, experience with pancreatic, bone-marrow, and heart-lung transplants. The value of cyclosporine in the treatment of autoimmune disease and disorders associated with altered immune reactivity has not been established and is currently under investigation. There are provocative suggestions that the requirements for insulin in insulin-dependent diabetes can be reduced substantially by cyclosporine, especially when drug treatment is initiated within 6 weeks of the onset of the disease.

The major toxic manifestations of cyclosporine are renal. Plasma concentrations of creatinine are usually elevated during therapy and require careful monitoring. Hepatotoxicity has been noted, and hepatic function should be assessed carefully. As with other immunosuppressive agents, there may be increased susceptibility to infection and the development of lymphomas. These lymphomas are associated with reactivation of the Epstein-Barr virus and are frequently similar to Burkitt's lymphoma (Kahan *et al.*, 1983; Thomson, 1983; Weil, 1984).

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