TWENTY-SECOND HAHNEMANN SYMPOSIUM

Cancer Chemotherapy II

Edited by

ISADORE BRODSKY, M.D.

and S. BENHAM KAHN, M.D.

Under the general editorship of

JOHN H. MOYER, M.D.

CANCER CHEMOTHERAPY II

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Preface

Additional knowledge has accumulated rapidly in cancer chemotherapy since our first symposium in 1965. Our purpose in conducting this second symposium on cancer chemotherapy was to update the first. Again our format has been to survey the field, presenting broad concepts but excluding controversy where it had not changed overall direction.

Several metastatic diseases other than malignant trophoblastic disease are now curable by chemotherapy. About 25 per cent of patients with Burkitt's lymphoma are cured by alkylating agents, whereas a smaller percentage of patients with metastatic testicular cancer and some patients with childhood lymphoblastic leukemia have been apparently cured by combination chemotherapy. Recently the demonstration that L-asparaginase, an enzyme, can inhibit certain leukemic cells while not impairing normal marrow cells has stimulated hope that a "magic bullet" for cancer might be found. However, as of this time the backbone of chemotherapy remains those agents whose cytotoxic capacity extends to normal cells as well as to tumor cells.

The use of combination chemotherapy in short repetitive courses, taking advantage of the fact that normal cells recover faster than malignant cells, has led to some spectacular results, especially in the therapy of Hodgkin's disease. Currently available data suggest that with this kind of treatment about 40 per cent of patients with Stage IV Hodgkin's disease will remain free of disease for 5 years.

The application of immunologic concepts to the therapy of malignancy has led to some interesting advances in cancer therapy. Patients with acute leukemia may have extended survival if prolonged stimulation of the immune system is undertaken with bacille Calmette-Guérin (BCG). The induction of interferon by nonspecific chemical agents is another approach in our efforts to bolster the body's reaction to the presence of malignant disease. These advances alone might mandate another text, yet none have basically changed our direction, although our path has been broadened and our energy revived.

This volume, like the first, groups the papers into five areas. First, the mechanism of action of the chemotherapeutic agents is covered in broad detail. This section has been expanded to include discussions of interferon, immunology, intermediary metabolism and hemostasis in relation to chemotherapy.

Second, the clinical management of the patient with neoplastic disease is extensively covered in ensuing chapters. Although they have recommended some dosages, most authors have stressed the concept of individualized courses, depending on such factors as the staging and aggressiveness of the tumor in specific patients. Again, since this form of therapy is for the most part palliative, treatment is approached with an investigative emphasis rather than with an established routine. This section has been expanded to include a clinical chemotherapeutic approach to brain tumor and skin cancer.

Third, an appreciation of bone marrow function and mechanisms for controlling marrow toxicity induced by use of these drugs is essential for the clinician. We have added a chapter on bone marrow transplantation. The chapters dealing with hematologic and infectious implications have been expanded.

Fourth, we have continued to emphasize the hematologic neoplasms, both because of our own interest in these diseases and because the currently available agents are

effective in controlling these disorders as well as in producing profound toxic effects on the hematopoietic system. Chemotherapy of hematopoietic disease continues to serve as a yardstick by which success of solid tumor chemotherapy must be measured.

In the last segment, we cover regional techniques in the management of metastatic cancer. In this area we could not exclude the use of X-ray therapy in the management of Hodgkin's disease. Our purpose here was to establish appropriate criteria by which we could measure the results of chemotherapy of this disorder. The problem of how far to go with X-ray therapy in Hodgkin's disease remains unsolved, but it is our hope that the reader will be able to evaluate more objectively the continuously appearing reports involving various modes of therapy of this disease.

It is obvious that our greatest deficiencies in chemotherapy exist in the management of the extremely common neoplastic diseases, such as lung, breast, colon, and cervical carcinoma. However, the successes in the management of the hematologic neoplasms must serve as guidelines for continued investigations of newer approaches in the therapy of the common, presently resistant neoplasms. Therapeutic nihilism will lead to an abandonment of newer approaches and, what is even more tragic, an excuse for abandoning the patient. We feel confident that perhaps in the next volume we shall be able to report on chemotherapeutic modalities that may lead to the cure of these diseases.

Finally, we would like to stress what is well known to the oncologist but frequently confusing to the layman and even to general physicians: Cancer is not a single disease, and therapy must be individualized and broken down into component parts. The physician who truly recognizes his responsibility to his patient with metastatic disease will view even present-day cancer chemotherapy as one of the great advances of modern medicine.

The editors wish to thank Mrs. Sage Cordell, Mrs. Edith Schwager, Mrs. Kathleen Sullivan, Mrs. Rita Lavner, and Mrs. Marie Smith, who helped so much in the preparation of this symposium and this volume.

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Philadelphia, Pa.

CONTENTS

III. Bone Marrow Physiology and Protection

IV. Hematologic Neoplasms

V. Regional Techniques

I. MECHANISM OF ACTION OF CHEMOTHERAPEUTIC DRUGS

Mechanism of Action of Alkylating Agents

By DAVID B. LUDLUM, PH.D., M.D.

THE ALKYLATING AGENTS as a group contain some of our oldest effective drugs for the treatment of cancer. Although their mechanism of action has not been completely elucidated, it is known that they alkylate nucleic acids and interfere with DNA synthesis in vivo. Furthermore, these agents can be either mutagenic or carcinogenic under certain conditions. This suggests that an attack on the genetic apparatus, most likely DNA, is basic to their action.

Therefore, to limit this discussion to a manageable size, we shall consider reactions of alkylating agents with nucleic acids and nucleic acid models only, and not cover their reactions with other cellular constituents. In a review of this size, many careful investigations cannot even be mentioned, but most of these are covered in other recent reviews and collected works.[1-10]

We shall consider the relevant chemistry of alkylation, certain biologic effects of alkylation, and biochemical studies which utilize synthetic polynucleotides as models for nucleic acids. Throughout, we shall be particularly interested in studies which relate to the two major clinical deficiencies of these agents: their incomplete selectivity for neoplastic tissue, and the development of resistance.

CHEMISTRY OF ALKYLATION

Representative alkylating agents are shown in Figure 1. These compounds are attacked by nucleophilic sites on nucleic acids and other cellular constituents; this results in an addition of a portion of the alkylating agent to the nucleophilic site. Extensive studies by Brookes and Lawley[5,11,12] and other investigators have identified the base positions shown in Figure 2 as primary sites of reaction. The nucleosides shown in this figure are from RNA, but the corresponding positions are involved in DNA. Because of experimental difficulties, there is less agreement about the extent of alkylation of phosphate groups along the nucleic acid backbone; however, recent experiments with model compounds[13,14] have indicated that some phosphate alkylation probably occurs.

Considerable interest has centered on substitution of guanine in the 7 position, a reaction which frequently accounts for more than 90 per cent of the total alkylation. It has been suggested that the 7-substituted base might mispair with thymine or uracil in nucleic acid replication or transcription (see, however, the discussion below in "Biologic Effects of Alkylation"); alternatively, following depurination of DNA at this site, any base might be introduced opposite the empty location in subsequent replica-

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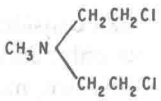
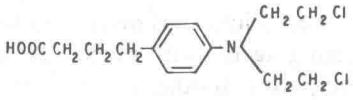
tive processes. More recently, certain genetic and biochemical studies have focused attention on the effects of alkylation at other sites, particularly the O-6 position of guanine[15] and the 3 position of cytosine.[16,17] Interest in reaction at these sites has been enhanced by the finding that agents with rather different biologic effects[18,19] may attack different base positions somewhat selectively under certain conditions.

It is an old observation that monofunctional agents generally have little therapeutic value, and that effective agents are found among compounds with two or more alkylating groups. These compounds can form crosslinks within a single molecule, or bind two macromolecules together with a relatively stable covalent bond. The existence of such links between the two strands of DNA may be demonstrated by the reversible denaturation of double-stranded DNA molecules.[20,21] Crosslinks seem to play an important role in explaining the action of many alkylating agents.

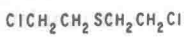
Apparent exceptions to the rule that clinically useful agents contain two or more



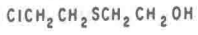
Nitrogen Mustard *



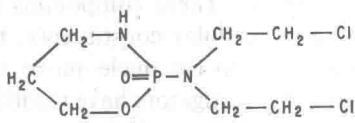
Chlorambucil *



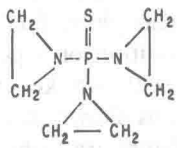
Sulfur Mustard



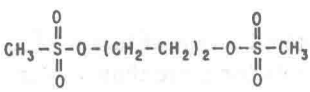
Half Sulfur Mustard



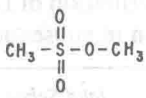
Cyclophosphamide *



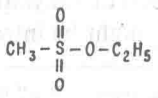
Triethylenethiophosphoramide *



Busulfan *



Methyl Methanesulfonate



Ethyl Methanesulfonate

FIG. 1.—Representative alkylating agents. The compounds indicated by an asterisk are in clinical use; the rest are of theoretical interest only.

alkylating groups are CCNU, 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea, and similar nitroso compounds which contain a single 2-chloroethyl group. However, it may be that the 2-chloroethyl group is transferred to a nucleophilic site and then acts as an alkylating group to produce a crosslink.

Although the main chemical features of alkylation have been elucidated, specific details of the process may be very important. Lawley and Brookes[12] have shown that the secondary structure of DNA influences the site of alkylation; studies with polynucleotides[22] have shown that this influence is very marked indeed. Changes in secondary structure during replication may well explain the special sensitivity of replicating cells to alkylation,[22,23] and more work should be done in this area.

Biologic Effects of Alkylation

Microorganisms, especially bacteriophages, are excellent models with which to study the lethal effects of alkylation. It has been shown[4,24,25] that bacteriophages which contain double-stranded nucleic acids are more sensitive to the action of difunctional agents than those which contain single-stranded material. This suggests that interstrand crosslinks are important in producing a lethal effect. However, even bacteriophages which contain single-stranded nucleic acids are more sensitive to difunctional than to monofunctional agents. This implies that intrastrand links are also important in explaining lethality. The studies described below suggest that somewhat different factors govern the lethality of different compounds; however, the clinically useful agents probably depend on crosslinking for their lethality.

Lawley and coworkers[21] have published a detailed study of the action of sulphur mustard and half mustard on the T7 coliphage, a virus which contains double-stranded DNA. At the mean lethal dose for immediate inactivation by mustard gas, there were about 1.3 moles of di-(guanin-7-yl-ethyl) sulfide and 7 moles of monoalkylation products per mole of DNA polymer, whereas at the mean lethal dose for half mustard there were 280 moles of monoalkylation products. These data indicate that formation of the crosslinked diguaninyl product is a particularly damaging event. Approxi-

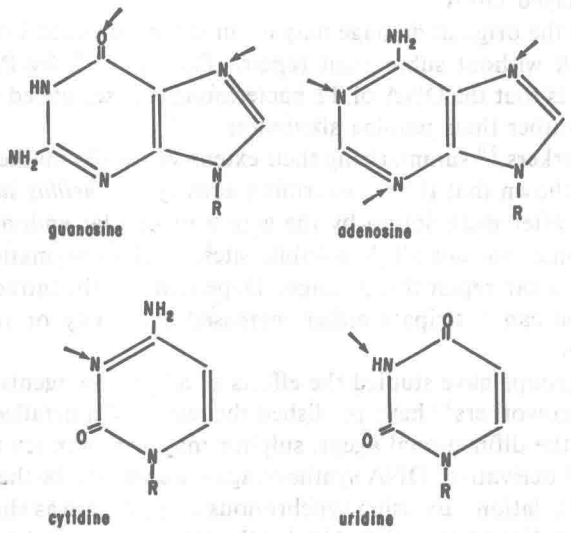


FIG. 2.—Reactive sites in nucleosides. The 7 position of guanosine is by far the most reactive.

mately one quarter of the diguaninyl molecules are associated with interstrand crosslinks and the remainder with intrastrand links.

A relatively large number of alkylations were required for immediate inactivation by the monofunctional half mustard. Subsequent depurination and hydrolysis of DNA at alkylated base positions caused more extensive inactivation than alkylation itself.

Verly and Brakier[26] have studied the action of nitrogen mustard, ethyl methanesulfonate, busulfan, and diepoxybutane on the same T7 phage. Crosslinks were again associated with lethality for nitrogen mustard; when survival was followed up after treatment with this agent, bonds between DNA strands first increased in number and then decreased.

On the other hand, treatment of T7 with ethyl methanesulfonate resulted in lethality which increased steadily after exposure, presumably due to depurination and hydrolysis. Surprisingly, when T7 was treated with the well-known difunctional agent, busulfan, there was no evidence for interstrand crosslinking. Intrastrand crosslinks were not ruled out, but the concentrations of busulfan and ethyl methanesulfonate required for equal lethality were similar. It thus appears that ethyl methanesulfonate and busulfan have a very similar action on T7 phage; some additional factor must operate to explain the difference between these two agents in higher organisms. The final agent studied, diepoxybutane, had a combined difunctional and monofunctional action.

When the effects of alkylating agents are studied in growing bacteria or mammalian cells, intracellular enzymes become extremely important in modifying the initial damage to DNA. These enzymes evidently recognize the alkylated base and remove it, an action which can either magnify the damage or open the way for subsequent repair.

Papirmeister and Davison[27] first demonstrated the loss of sulphur mustard alkylation products from the DNA of *Escherichia coli*. Bacteria which were treated with this agent and then incubated under certain conditions gradually regained their ability to synthesize DNA and to reproduce after an initial lag. Since sulphur mustard products were eliminated during this period, it was assumed that growth returned only after repair of damaged DNA.

Magnification of the original damage may occur if the alkylated DNA is subject to endonuclease attack without subsequent repair. Recent work by Papirmeister and coworkers[28] suggests that the DNA of T1 bacteriophage is sensitized to endonuclease attack by adenine rather than guanine alkylation.

Strauss and coworkers,[29] summarizing their extensive studies on the effects of DNA methylation, have shown that the transforming activity of *Bacillus subtilis* DNA can be greatly reduced after methylation by the action of cellular endonucleases. These nucleases attack some, but not all, heat-labile sites. Other enzymatic activities have been identified which can repair this damage. Depending on the intracellular environment, therefore, we can anticipate either increased sensitivity or resistance to the effects of alkylation.

Many different groups have studied the effects of alkylating agents on mammalian cells; Roberts and coworkers[30] have published the results of a detailed study of HeLa cells. Here again, the difunctional agent, sulphur mustard, is much more toxic than the monofunctional derivative; DNA synthesis again appears to be the cellular process most sensitive to alkylation. By using synchronous cultures, it was shown that cells in the late G_1 (postmitotic) or early S (DNA synthetic) phases are particularly sensitive to the effects of alkylation.

When cells are treated during these phases, there is an immediate inhibition of DNA synthesis and a delay in division and onset of the next (second) DNA synthetic cycle. However, it is evident that some recovery has occurred by the third synthetic cycle. This is attributed to repair of alkylated DNA, a process which is accompanied by release of alkylated products. Thus, the same general features of cytotoxicity appear to be present in both mammalian and bacterial cells.

The experiments described above are concerned, first, with the lethal action of alkylating agents, and then with the modifications of lethality which are imposed by cellular metabolism. Another approach, the genetic study of point mutations, yields useful information on the nature of the original DNA lesion produced by alkylating agents. Since the structure of the mutated DNA cannot be determined directly, the chemical identity of the altered base must be deduced from the altered gene product, or from genetic reversion data.

Tessman and coworkers[31] studied the effects of an ethylating agent, ethyl methanesulfonate, on a single-stranded DNA phage, S13. These data showed that changes in all four bases could occur, although one change appeared most frequently. They assigned this to a GC → AT transition, which seemed reasonable since guanine is, of course, the most readily alkylated base. The earlier data of Bautz and Freese,[32] as well as more recent studies by Osborn and coworkers,[33] support this hypothesis, but studies of amino acid replacement in the A protein of tryptophan synthetase[34] did not result in a clear-cut assignment. Thus, there is some uncertainty as to the base changes produced by alkylation, and the fact that all four bases can evidently be altered should be emphasized.

The problem of relating a particular alteration in nucleic acid structure to a specific biologic effect is greatly simplified by the use of synthetic polynucleotides.[35] These polymers may be prepared with a known composition and used in model biochemical systems to obtain direct information on the biologic effects of alkylation.

Systems which are available for such studies and which are part of the mechanism for transferring genetic information are shown in Table 1. Each of these systems utilizes the informational content of the polymer at the left to direct the synthesis of the polymer at the right. The template is incubated with the required enzyme or cell extract, and the performance of an unalkylated template is compared with the performance of an alkylated one. The genetic damage associated with a particular base substitution can then be observed directly.

Because of their greater availability, experiments with polyribonucleotides have progressed further than experiments with polydeoxyribonucleotides. However, the base-pairing properties of synthetic polydeoxyribonucleotides have, in general, resembled those of the ribose series. Accordingly, one would expect abnormal base pairing behavior in one series of polymers to be reflected in the other series.

TABLE 1.—*Models for Testing the Effects of Alkylating Agents on the Properties of Templates*

System	*Enzyme*
DNA → DNA	DNA polymerase
DNA → RNA	RNA polymerase
RNA → RNA	RNA polymerase
RNA → protein	Cell extract