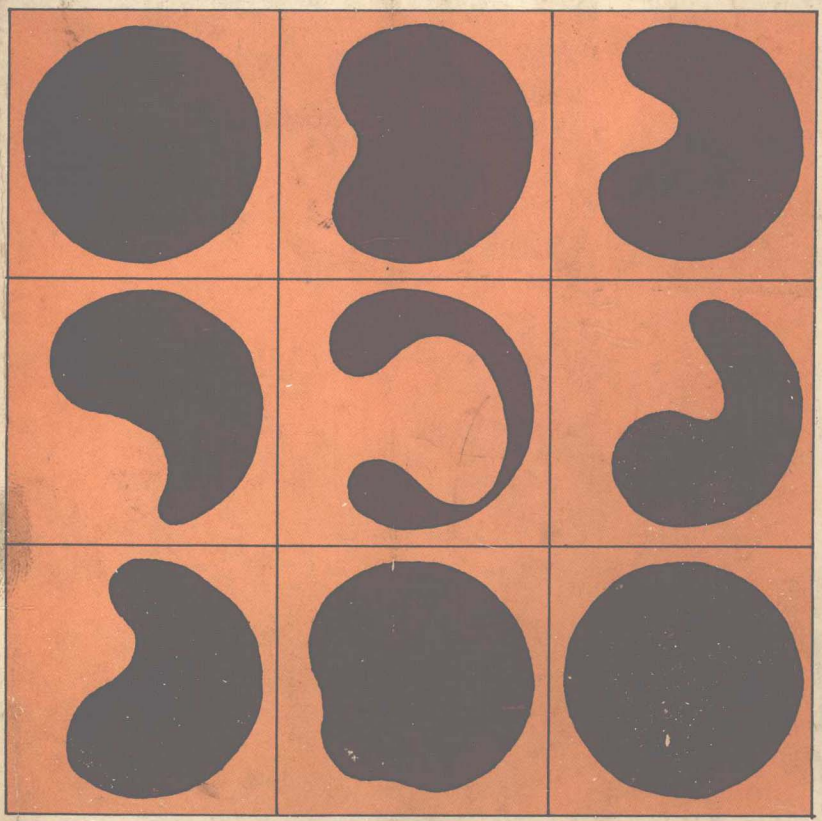
Drugs and the Cell Cycle



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Cell Biology/ A Series of Monographs

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Preface

In 1876 Tyndall reported that a species of penicillin exhibited action antagonistic to bacterial growth. It was not until 1940, however, that a preparation of solid penicillin was placed into the therapeutic arsenal of the medical world.

In this book an attempt is made to introduce fundamental principles and studies on the mechanisms of drug action on proliferating cells in an effort to reduce the time lag between observation and practical application. The subject matter reviewed will be of interest to investigators in many disciplines, particularly to physiologists, pharmacologists, and oncologists, as well as to those working in cellular, developmental, and molecular biology. This work should serve to bridge the gap between experimental laboratory observations and their potential relevance to mankind.

This volume is comprised of chapters dealing with plant alkaloids, alkylating agents, mercurials, adrenergic agents, radiomimetics, narcotics, hallucinogens, mitogens, hepatotoxins, antibiotics, and antimetabolites of various types. The drugs used in cancer chemotherapy are given special emphasis. Bacteria, protozoa, sea urchin, and mammalian cell systems are discussed. The mammalian cell studies deal with both *in vitro* and *in vivo* cell systems.

A great deal of information and current concepts are summarized in this book, and it is hoped that it will act as a stimulus for new research.

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Contents

1

Perspectives on Drugs and the Cell Cycle

IVAN L. CAMERON

A dictionary defines drugs as chemical substances that are given to people and animals as medicine. Certainly most of the chemical agents listed and discussed in this book have been tried as potential medicines and may qualify as drugs on this basis alone. This dictionary definition of drugs is inadequate for our discussion because it limits use of the term drugs to medicinal chemicals. The definition ignores the fact that many chemical agents commonly referred to as drugs are exceedingly useful tools and probes for working out metabolic pathways and cellular processes even though they have little or no direct medicinal value. We, therefore, choose to use the word drug in a broader sense.

Several of the chapters in this book bring together new and diversely scattered information about the action of cancer chemotherapeutic drugs on the cell cycle of normal and tumor cell populations. Other contributions illustrate how information on the effects of drugs on the cell cycle can give a better understanding of the sequence of events taking place in the cell cycle. Concurrently, these same cell cycle studies are giving a much better understanding of how a particular drug works.

I. Chemotherapy and the Cell Cycle

Much of what is presented in this book can be related to the concepts of chemotherapy. These concepts were first applied to antimicrobial agents by

Paul Ehrlich, who is recognized as the father of chemotherapy (Franklin and Snow, 1971). During the decade following 1902, Ehrlich established most of the concepts and principles from which subsequent work on chemotherapy has evolved. Although the principles of chemotherapy were well established by Ehrlich, the field had only limited practical success until the introduction of antibiotics in the late 1930's and early 1940's. It is really only since 1959, and the rapid accumulation of information on cell proliferation kinetics, that rational cancer chemotherapy has developed from an art to a science (for general reference, see Elkerbout *et al.,* 1971).

It has been an objective of chemotherapy to describe the mode of action of a specific drug as related to its biological effects on sensitive cells and to describe in molecular detail the interaction between the inhibitor (drug) and its target or receptor within the cell. This principle was stated by Ehrlich himself in 1909: "In order to pursue chemotherapy we must look for substances which possess a high affinity and high lethal potency in relation to the parasites, as selectively as possible. In other words, we must learn to aim and to aim in a chemical sense." (The term parasite in this quotation may refer to viruses, to bacteria, to fungi, or to cancer cells.)

Using these principles, Ehrlich himself had some success in development of chemotherapeutic treatments. Among his therapeutic contributions was the synthesis of several organoarsenical compounds, one of which (Neosalvarsan) was used as the main treatment of syphilis until penicillin was produced. Another drug coming from Ehrlich's work was suramin, produced from trypan red, and used in the treatment of trypanosomiasis. His work also led to the use of a product of methylene blue called mepacrine (atabrine or quinacrine) which has antimalarial value.

The influence of chemotherapy had its most striking manifestations in the antibiotic revolution, which led to the development of penicillin and other antibiotic drugs such as those developed by the soil microbiologist Waksman in the 1940's. The influence of the antimicrobical drugs can be appreciated by an analysis of Table I. Here we see the 10 leading causes of death in the United States in the years 1900 and 1959. It can be seen that bacterial infections are involved in six of the ten main causes of death in 1900. After the development and application of antibiotics, only pneumonia remained as a bacterial infection among the ten leading causes of death.

Table II lists the mode and site of action of some common antibiotics. These antibiotics are isolated as substances elaborated by various microorganisms and, in low concentration, inhibit the growth of other cells or microorganisms. A perusal of the table will indicate that the various agents have rather specific sites of action in cells. Perhaps the best known of the antibiotics on this list are the penicillins. Penicillins interfere with cell wall

TABLE I

THE TEN LEADING CAUSES OF DEATH IN THE UNITED STATES IN 1900 AND 1959[a]

Rank (1900)	Cause of death	Percent of deaths from all causes	Rank (1959)	Cause of death	Percent of deaths from all causes
1	Pneumonia and influenza	11.8	1	Diseases of the heart	38.6
2	Tuberculosis	11.3	2	Cancer and other malignancies	15.7
3	Diarrhea and enteritis	8.3	3	Cerebral hemorrhage	11.5
4	Diseases of the heart	8.0	4	Accidents	5.4
5	Cerebral hemorrhage	6.2	5	Certain diseases of early infancy	4.1
6	Nephritis	5.2	6	Pneumonia and influenza	3.5
7	Accidents	4.2	7	General arteriosclerosis	2.1
8	Cancer	3.7	8	Diabetes mellitus	1.7
9	Diphtheria	2.3	9	Congenital malformations	1.3
10	Meningitis	2.0	10	Cirrhosis of liver	1.2

[a] Modified after Strehler (1962).

TABLE II

MODE AND SITE OF ACTION OF SOME ANTIBIOTICS

Antibiotic	Action
Penicillins and cycloserine	Interferes with cell wall (murein) synthesis
Azaserine and DON	Blocks *de novo* synthesis of purine nucleotides
Mitomycin	Cross-linking of DNA strands
Actinomycin D	Suppress DNA-dependent RNA synthesis probably by binding double-stranded DNA or by intercalating into DNA
Rifamycin	Inhibits bacterial RNA polymerase
Puromycin	Prematurely terminates growing peptide chain on 70 S or 80 S ribosome
Streptomycin	Inhibition of initiation complex formation and transfer RNA-ribosome interaction works specificially on 70 S ribosomes
Tetracyclines	Inhibits binding of aminoacyl-tRNA to acceptor site on both 70 S and 80 S ribosomes
Chloramphenicol and erythromycin	Inhibitors of peptide bond formation and translocation on 70 S ribosomes
Cycloheximide	Specifically inhibits function of 80 S ribosome
Sulfanilamide	Interferes with folic acid synthesis in bacteria; mimics *p*-aminobenzoic acid
Methotrexate	Inhibits folic reductase
Antimycin	Blocks respiratory chain immediately before cytochrome c_1
Oligomycin	Interferes with oxidative phosphorylation

production in bacteria. This inhibition produces a cytostatic effect on growing bacteria.

A good example of the relationship between the action of the drugs and the cell cycle is illustrated by the action that penicillin has on growing bacteria. It is thought that the sole bacterial chromosome replicates during the cell cycle and that the two resulting chromosomes are attached in some way to the cell membrane. The newly replicated bacterial chromosomes are then distributed to daughter cells by means of growth of the cell membrane between the points of attachment of the chromosomes. This separates physically the two bacterial chromosomes and starts the next cell cycle. Penicillin interferes with the growth of the cell wall material and, therefore, interferes with membrane expansion and the separation of the bacterial chromosomes. The drug does not actually kill the cell directly but simply interferes with the formation of the cell wall and cell reproduction.

Animal cells, which do not possess cell walls, are not affected by this drug. This difference between bacteria and animal cells is the basis of the selectivity of penicillin action. Likewise, bacteria in a spore state are not affected by the drug because no new cell wall synthesis is occurring. On the other hand, those bacteria in a growth state will have a weakened cell wall which can then be attacked by cytocidal agents, such as phenol or hypotonic solutions. A weakened cell wall cannot, for instance, keep the cell from swelling and rupturing in hypotonic solutions.

Among the list of compounds in Table II are other antibiotics that possess specificity of action. For example, rifamycin inhibits specifically bacterial RNA polymerase. Streptomycin, chloramphenicol, and erythromycin act to inhibit protein synthesis which specifically involves 70 S ribosomes. Eukaryotic cells having 80 S ribosomes in their cytoplasm are generally not adversely affected by these antibiotics. On the other hand, cycloheximide specifically inhibits the function of 80 S ribosomes in eukaryotic cells. It seems important to mention that mitochondria and chloroplasts of eukaryotic cells also contain 70 S ribosomes, whereas the rest of the eukaryotic cell has 80 S ribosomes. This accounts for the fact that it is possible to selectively inhibit chloroplast reproduction and, therefore, to bleach the chloroplasts from the eukaryotic cell, *Euglena,* by streptomycin treatment. However, *Euglena* is still able to grow and reproduce if additional nutrient supplements are added to the growth media. For similar reasons we may be able to account for the failure of lymphoid cells to produce antibodies when the cells have been treated with chloramphenicol and to explain the observed deficiency of cytochrome c reductase in rat heart cells cultured with chloramphenicol. Presumably, in these latter cases, the drug is preferentially acting on the 70 S ribosomes of mitochondria. Thus, it appears that treatment of bacterial infections with some inhibitors of protein synthesis may depend on the specificity of attack on the 70 S ribosome of bacteria, leaving the 80 S predominant ribosome of the host organism unaffected. In cases of those protein-inhibiting drugs that affect both the 70 S and 80 S ribosomes, such as puromycin and the tetracyclines, a differential permeability of the drugs into the bacteria may account for the specificity of inhibitory action.

Clearly, then, chemotherapy takes advantage of the chemical differences existing between the parasite and the host. Such differences are readily apparent between bacteria and the animal cell, but what are the differences between normal animal cells and cancer cells? Unfortunately, few differences are now known (Elkerbout *et al.,* 1971). Introduction of the tools needed for the study of cell proliferation kinetics *in vivo* and *in vitro* have led us to realize that there are at least some small differences in the cytokinetics of some cancer cell populations in comparison to the cytokinetics in

the normal cell populations of the hosts. These cytokinetic studies have led to the recognition that a few specific types of cancer are rapidly proliferating and that most of the cancer cells are in the proliferative state (have a high growth fraction). In man, these rapidly proliferating cancers include choriocarcinoma, Burkett's tumor, Hodgkin's disease, acute lymphocytic leukemia, and Wilm's tumor. A list of some drugs used in cancer chemotherapy is given in Table III. A detailed review of action of some of these drugs can be found in the chapter by Wheeler and Simpson-Herren (this volume). These drugs clearly have selective effectiveness for proliferating cells. Table III

TABLE III

THE ACTION AND CELL CYCLE PHASE SPECIFICITY OF SOME CANCER CHEMOTHERAPEUTIC DRUGS[a]

Drug	Action and end product affected	Cell cycle phase specificity
Cytosine arabinoside	Inhibition of nucleotide reductase (DNA)	S-phase specific
Hydroxyurea	Inhibition of nucleotide reductase (DNA)	S-phase specific
Guanozole	Inhibition of nucleotide reductase (DNA)	S-phase specific
Methotrexate	Inhibits folic reductase (DNA, RNA, protein)	S-phase specific but self-limiting[b]
6-Mercaptopurine	Inhibits PRPP→phosphoribosylamine (DNA, RNA)	S-phase specific but self-limiting[b]
5-Fluorouracil	Inhibits thymidylate synthetase, incorporates into RNA (DNA, RNA)	S-phase specific but self-limiting[b]
Cyclophosphamide and 1,3-bis(2-chlorethyl)-1-nitrosourea (BCNU)	Cross-links DNA strands	Specific for proliferating cells, especially those cells lacking DNA repair enzymes
Vinblastine and vincristine	Inhibits assembly of microtubular proteins into microtubules	Mitosis
Cytochalasin B	Inhibits function of microfilaments	Cytokinesis
Actinomycin D	Inhibits DNA dependent RNA synthesis	Not considered cell cycle specific but may have some selectivity for proliferating cells

[a] Modified after Skipper *et al.* (1970).

[b] These drugs tend to retard proliferating cells not in S phase at the time of drug application from progressing to S phase.

suggests that an optimal schedule for S-phase specific drugs would have to reach effective serum levels of the drug at intervals of just less than the S-phase duration if all the proliferating tumor cells are to be affected. Skipper *et al.* (1970) have indeed proved this scheduling to be the most effective for the S-phase specific drugs; thus, as these authors point out, optimal drug scheduling can make the difference between failure and success. It is to the credit of modern cancer chemotherapy that the five types of rapidly proliferating human cancer mentioned above are now successfully managed, if not cured.

It is a paradox that among people with cancer those with the five specific types of cancer mentioned above formerly had the worst prognosis for long-term survival, but now they have the best prognosis. It is quite evident that improved quantitation and better fundamental information concerning the pharmacology, the toxicology, the cell-proliferation kinetics, and the cellular response of chemotherapeutic agents will continue to aid clinicians in planning therapeutic regimens.

Just as the drugs listed in Table III inhibit proliferating cells of cancer cell populations they also play havoc with proliferating cell populations of the host tissues. These proliferating cell populations include those of the bone marrow, the lymphatic tissues, the linings of the gastrointestinal tract, the epidermis, and other rapidly proliferating cell systems within the body. Continual use of these chemotherapeutic agents can be expected to lead to such cytostatic side effects as would be predicted by interference with cell reproduction in such cell populations. Some of the most adverse effects are brought about by interfering with megakaryocyte proliferation, which causes loss of blood platelets leading to hemorrhage and hemophilia. The loss of production of red blood cells leads to anemia. Interference with lymphocyte, granulocyte, and plasmocyte production causes suppression of the body's defense mechanisms, which normally operate against the spread of invading microorganisms, and also causes the suppression of the body's immunological system.

These drugs cause loss of the linings of the gastrointestinal tract, resulting in stomatitis, diarrhea, and vomiting. One can also expect that inhibition of cell proliferation in the epidermis will lead to the dermatitis, loss of hair, etc. In males sperm production is disturbed; in women, amenorrhea may result. The rapid rates of cell proliferation in embryonic cell populations make the embryo a prime target for drug action. The result of the use of such chemotherapeutic drugs during pregnancy is teratogenic deformations and embryonic death.

It is, therefore, clear that if we are to use these highly toxic agents we must know and exploit the subtle differences that exist between normal and tumor cytokinetics. Some of the contributions to this book give new and

useful information on the action of cancer chemotherapeutic drugs on the mammalian cell cycle of normal and tumor cell populations (see the chapters by Hagemann and Lesher and by Hoffman and Post in this volume).

Looking at the problem of cancer chemotherapy from the cytokinetics point of view leads one to the conclusion that the slow-growing tumor, which contains cells with long cell cycle times and, with many cells no longer in the cell cycle at all, will be difficult or impossible to manage or cure.

We should not, however, be detracted from trying to find other possible differences between normal and neoplastic cells, which can be exploited as a basis for selectivity of drug action. Some of these differences may include (1) sensitization of breast and prostatic cancer by particular sex hormones; (2) the selective accumulation of cytotoxic agents by target tissues, such as occurs in the case of radioiodine in the thyroid; (3) the selective activation of drugs by target tissues, such as the activation of cyclophosphamide by those tissues rich in phosphamidase; (4) taking advantage of the particular differences in metabolism, such as starving some of the asparagine requiring leukemic cancers by asparaginase treatment to remove asparagine from the serum; (5) the use of antimetabolites against specific metabolic requirements; (6) the continued development of combination therapy, which overcomes the problem of the tumor cells developing resistance to the action of one drug; or (7) the introduction of carrier molecules onto carcinostatic agents to increase the permeability and effectiveness of the drug, as in the case of uracil mustards. Of course it also seems probable that some of the tumors caused by viral infections will be subject to direct chemotherapeutic attack or to treatment by established immunization techniques.

More studies need to be conducted to determine means for "priming," "setting up," or "synchronization" of tumor cells *in vivo* for chemotherapeutic attack. Such possibilities include starvation and refeeding experiments, use and enforcement of normal diurnal rhythms of drug susceptability, and surgical removal of a large tumor mass, which would stimulate increased cell proliferation of the tumor cells in metastatic sites. Establishing the existence and isolation of tissue or tumor specific factors, such as the tissue specific chalones, or perhaps introducing specific mitogenic materials to stimulate specific types of tumors may also prove rewarding in priming tumor cells for drug therapy. For a further discussion of the nature and action of such tissue specific mitogenic agents the reader is referred to the chapter by Cooper in this volume.

II. The Cell Cycle as a Sensitive Indicator for Drug Analysis

In the United States we are faced daily with the question of the possible dangerous effects of narcotics and hallucinogenic drugs. We hear politicians