Some Metabolic Approaches to Cancer Chemotherapy*

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INTRODUCTION

The title of this article may imply that the present state of metabolic knowledge is now sufficient to permit the successful design of new agents for the chemotherapy of cancer. However, the complete acceptance of such a view is not yet possible; in fact, the word "approaches" in the title signifies that the desired metabolic goal is still being sought. Indeed, we cannot yet be certain that satisfactory control of the many types of neoplastic diseases through the use of drugs is an attainable goal. Let us hope, however, that our present state of relative ignorance does not indicate that we are in the frustrating predicament of the individual who, having great difficulty in describing a suitable route to an isolated area, finally concluded that you just can't get there from here!

What, then, is the rationale of some of the present metabolic approaches to cancer chemotherapy, both theoretical and actual?

To comment first about the theoretical: it would appear that the most rational approach should be based on an essential biochemical difference of a qualitative nature between neoplastic cells and their normal counterparts. Thus, the marked successes which have occurred in the chemotherapy of a variety of infectious diseases caused by parasitic microorganisms have resulted from the development, either empirically or occasionally by design, of agents which interfere with biochemical reactions that are essential to the life of the invading organisms but have little or no significance to the mammalian host. Unfortunately, analogous qualitative differences have not appeared to characterize neoplastic cells, as compared with normal cells. Indeed, in the process of the probably gradual conversion of a normal parent cell to a malignant descendant, one may inquire whether it is logical to anticipate that new chemical reactions should appear which are essential to the continued existence of the neoplastic state. Although it is possible that such critical acquisitions may occur, they have not yet been found, and it is perhaps more likely that in neoplastic cells various biochemical features characteristic of the parent cell may have been deleted and that such cells characteristically have lost the capacity to respond to various environmental controlling influences—about which more will be said subsequently. At present, however, it is important to note that a few biochemical changes of a quantitative nature are not uncommonly encountered in tumors, as compared with normal tissues. The one most commonly cited, of course, is the remarkable capacity of many types of malignant tissues to carry on aerobic as well.

* This article is based on a series of lectures, presented for the American Cancer Society, at the following universities: Arizona State (Tempe), Florida (Gainesville), Iowa (Iowa City), Oregon State (Corvallis), and Wake Forest (Winston-Salem); and, under other auspices, at the Universities of Colorado (Denver), Manitoba (Winnipeg), and Washington (Seattle), at Vanderbilt University (Nashville), and at the Annual Arizona Cancer Seminar (Tucson). Work in the laboratories at Yale was supported by a grant (CY-2817) from the National Cancer Institute, U.S. Public Health Service, and by grants (T-17 and T-28) from the American Cancer Society.

Received for publication July 20, 1961.
as anaerobic glycolysis at sustained high levels, with the production of large amounts of lactic acid from glucose. However, not only is this feature not always observed in tumors, but also some normal tissues can exhibit higher values than tumors; certainly, the meaning of this phenomenon has not yet been explained satisfactorily.

Relatively little hope for selective attack upon the cancer cell is to be anticipated from attempts to disturb the three major pathways of energy-release within cells—that is, glycolysis, the tricarboxylic acid cycle, and the respiratory cycle, since, although their relative efficiency and control may be altered in tumors, the reactions of these adenosine triphosphate (ATP)-producing systems are fundamental to all mammalian cells, and significant inhibition of any of the many enzymes concerned with these systems almost certainly would have grave effects on normal cells.

Although some neoplastic cells are highly differentiated and may carry out specialized metabolic reactions characteristic of the cells of origin, none of these reactions appears to be essential either to the continued reproductive existence of cells or to their retention of the properties of malignancy. Accordingly, an attempt to interfere with such specialized functions, if and when they are present in malignant cells, has not appeared to be a particularly promising area for investigation.

If we are to view with pessimism the search for biochemical reactions unique to cancer cells, which could be inhibited in tumors with selective effects upon them, we may ask what approaches remain?

Two important possibilities seem to be afforded at the present time. One of these, not yet investigated to any great extent, would attempt to exploit enzymic deficiencies or deletions of certain neoplastic cells. In some cases, at least, these deficiencies appear to place the aberrant cell at such a biochemical disadvantage that unique opportunities may be afforded for chemotherapeutic attack. More concerning this approach will be presented in connection with a discussion of some specific mechanisms of drug resistance, a phenomenon now explicable, in a few instances at least, on the basis of specific enzymic deficiencies. However, it is preferable first to discuss the approach which is now in principal vogue—namely, the search for agents which may inhibit enzymically catalyzed reactions essential to cell reproduction, especially those concerned with the formation or utilization of precursors of nucleic acids.

Clearly, the hope for finding agents with real selectivity through such an approach would appear to be small. The possibility of attaining specific interference with such mechanisms only in neoplastic cells has seemed almost too much to hope for, since the chemical reactions of cell multiplication do not appear to differ qualitatively from those characteristic of normal cells. Nevertheless, several drugs have been developed which may produce, at least for a time, remarkably beneficial effects. These effects may be attributed to the slightly greater susceptibilities of some types of malignant cells, as compared with the susceptibilities of such rapidly reproducing normal cells as occur, for instance, in the epithelium of the alimentary tract and in the bone marrow.

It may be emphasized that it is no longer justifiable to contemplate with customary pessimism the possibility of obtaining chemotherapeutic agents which can act selectively upon neoplastic cells. This is because at least one compound, 6-azauridine, has been developed which produces no apparent deleterious effect upon any normal cells of man, but exerts a lethal effect, at least for a while, upon a few types of human leukemic cells. Although more will be said subsequently about this new agent and its potentialities for chemotherapeutic application, the importance of its mention at this point is to dispel the attitude of defeatism which often clouds a discussion of metabolic approaches to the rational development of selective agents for the chemotherapy of neoplastic diseases. Azauridine may not prove to be even a partial answer to the effective, long-term control of any form of neoplastic disease, but its existence certainly affords a basis for hope that the goal of selective attack upon cancer cells may not be unattainable.

INTERFERENCE WITH CELLULAR REPRODUCTION

It would be impossible in a brief article to discuss, except in a most cursory manner, the several compounds which have been developed for, or have proved to act by, interrupting one or another phase in the complex processes of cellular reproduction, either animal or bacterial. In many cases, detailed knowledge of the metabolic step interfered with is still being sought or only retroactively has become available. Despite this, however, in much the same manner that we learn better how to treat disease in man by conducting thorough autopsies, new potential metabolic approaches to cancer chemotherapy may be afforded by examinations of the attributes and disadvantages of chemical agents which have proved to have some utility. And, although little can be said here about compounds which have not proved
useful, it should be emphasized that there is often much to be gained from attempts to account for the relative inactivity of compounds originally thought to have promise.

About two decades ago it became appreciated, mainly as a result of studies of the mechanism of action of the antibacterial sulfonamides, which interfere with the utilization of p-aminobenzoic acid, that specific antagonists of naturally occurring substances can often be produced by making relatively small changes in the structure of metabolites. In this manner, metabolic antagonists of a great variety of important compounds, such as pantothenic acid, thiamine, nicotinic acid, pyridoxine, amino acids, etc., were prepared, and many of these have been studied extensively. Some of these compounds have been of value in the investigation of biological mechanisms, and heuristically, but only a very few have had important chemotherapeutic applications. The principal reason for this will be evident if it is realized that such antagonists are not likely to have selective effects if they interfere with the utilization of compounds critically involved in energy transfer, or in other basic cellular reactions, or in the structure of mammalian cells in general.

Concerning the mechanism of action of such antimetabolites, it may be sufficient here to refer to them as competitors for an enzyme which normally combines with and alters the corresponding metabolite. Thus, depending on the relative affinities of the two compounds for the specific enzyme, and on the relative concentrations which can be attained at the site of action, it is possible with many such agents to inhibit markedly the course of reactions involving the specific metabolite. Obviously, the effectiveness of such competitive antagonists in a tissue extract containing the specific enzyme, or even in cell culture, does not insure that activity will be observed in intact animals or man, since, to be useful in vivo, potential antimetabolites, in addition to exhibiting intrinsic activity, must satisfy a variety of other criteria related, for example, to their absorption, distribution, metabolic alteration and excretion, and to various possible side-effects. In addition, many examples exist of compounds which, although quite without inhibitory activity in vitro, acquire the desired antagonistic property after metabolic alteration.

Folic acid antagonists.—Perhaps the first antimetabolite to have real utility in the chemotherapy of neoplastic disease in man was a simple analog of pteroylglutamic (folic) acid—namely, aminopterin. When it became apparent that folic acid is critically involved in the reproduction of cells (89, 90) and indeed can increase the rate of cell proliferation in certain types of leukemia (52), a program of deliberate structural alteration was initiated, and many derivatives of pteroylglutamic acid were prepared (82). Of these, aminopterin, a compound in which a single alteration (substitution of an amino group for an hydroxyl group) had been made, and amethopterin ("Methotrexate") (an N-methyl derivative of aminopterin) emerged as compounds with tremendous antimetabolic activity; the latter is widely used in the treatment of the acute leukemias of children and certain other types of malignancy, particularly choriocarcinoma (31, 49, 53).

It is now possible to explain the action of these remarkably potent antimetabolites on the basis of their action on a specific enzyme, folic acid reductase (57, 65, 66, 68, 98). This enzyme is responsible for the reductive conversion of the vitamin, folic acid (F), to its unstable coenzyme form, tetrahydrofolic acid (FH4); the latter, in turn, is involved in a variety of one-carbon transfers essential to the life and reproduction of cells (49). Those transfers which are particularly concerned with cell reproduction are to be found in (a) the biosynthesis de novo of the first purine-containing compound, inosinic acid, which subsequently is converted into the adenine- and guanine-containing components of nucleic acids and many coenzymes, and (b) the conversion (with the aid of thymidylate synthetase) of deoxyuridylic acid into thymidylic acid, a constituent of deoxyribonucleic acid (DNA) containing the unique base, thymine. In these reactions, two derivatives of tetrahydrofolic acid are essential: (a) an N\textsubscript{5},N\textsubscript{10}-anhydroformyl derivative (\textsubscript{10}FH\textsubscript{4}), which can be regarded as an "active" formyl group (-CHO) and is required for the biosynthesis of the purine, and (b) an N\textsubscript{5},N\textsubscript{10}-methylene derivative (\textsubscript{10}FIL\textsubscript{4}), which behaves as an "active" one-carbon unit at the oxidation level of formaldehyde (CH\textsubscript{2}O) and is required in the enzymatic formation of thymidylic acid (49, 57, 74).

In order that these folic acid-derived intermediates be provided to cells, the unstable FH\textsubscript{4} must be formed enzymatically from either folic acid or dihydrofolic acid (FH\textsubscript{2}) or by the turnover of coenzyme molecules. Not only can the dihydrocompound be regarded as an intermediate in the formation of the coenzymatically active tetrahydrofolic acid, but it is also the end-product formed from \textsubscript{10}FIL\textsubscript{4} when deoxyuridylic acid is converted to thymidylic acid (35). Since the conversion of both folic acid and dihydrofolic acid to the tetrahydro-form is dependent upon folic acid reductase, and it is this enzyme upon which aminopterin...
of folic acid, it was once thought that it might be profoundly inhibited by these antimetabolites (49, 56).

So powerful is amethopterin as an antagonist of folic acid, it was once thought that it might combine irreversibly with the enzyme which modified folic acid and thus inactivate it (39). However, it is now known that the affinity of the enzyme for this antimetabolite, as compared with its affinities for the normal substrates (F and FH₂), is so great that it is difficult to demonstrate the competitive nature of the antagonism (94). Because of this remarkable affinity of folic acid reductase for amethopterin, the effects of the drug are not only long-lasting, but also impossible to overcome in vivo by giving large doses of folic acid. However, reversal of the early signs of toxicity of amethopterin follows the administration of a relatively stable derivative of FH₄—i.e., folinic acid (citrovorum factor, “CF”) (10, 12, 79, 81, 85), an agent which supplies the product of the blocked reaction but does not release the enzyme from its inhibition by amethopterin.

Studies with mouse leukemia cells grown in culture (34, 80) indicate that this type of cell uses folic acid quite inefficiently in comparison with folic acid, apparently because of inefficient uptake of folic acid and a barely adequate amount of folic acid reductase. As a result, the growth of such leukemia cells is very sensitive to inhibition by amethopterin (32). Recent evidence indicates that the chemotherapeutic efficacy of this antimetabolite in certain human leukemias, as well as in certain mouse leukemias, also is related to the supply of folic acid reductase in the drug-susceptible cells (4, 5). In view of the remarkable results obtained by Hertz and his colleagues with choriocarcinoma in the human female treated with amethopterin (39), studies of the folic acid reductase content of the cells of this neoplasm will be of much interest.

The efficacy of amethopterin in causing the rapid death of certain types of malignant leukocytes might appear to justify the expectation that the drug should be able to cure leukemia. However, it is well known that it does not do so in man, although in some cases it may induce excellent remissions; in mice, however, amethopterin can cure when the animals are inoculated with relatively small numbers of leukemia cells and rather promptly treated with the drug. It now appears that the capacity of this drug to cause only temporary remissions in certain leukemias of man is explainable, at least in part, at an enzymatic level (86). Thus, among the myriads of rapidly reproducing leukemia cells in a population, a few always seem to occur in which the level of folic acid reductase is higher than that which characterizes the bulk of the population. Such cells have too high a complement of this enzyme to be inhibited completely by amethopterin in concentrations which the normal cells of the body can tolerate. Unfortunately, this characteristic of producing larger amounts of folic acid reductase appears to be a manifestation of a genetically stable mutation. In other words, the cells high in enzyme survive in the presence of maximally tolerated extracellular concentrations of the drug and become the progenitors of a new population of leukemia cells which are similarly high in folic acid reductase and, therefore, are drug-resistant (32, 33). Recent studies with Sarcoma 180 cells in culture have given similar results (41), whereas investigations with human leukemic cells indicate that the conclusions drawn from work in mice may be transferable to man (6). It should be emphasized, however, that recent studies by Fischer¹ have shown that, in culture, murine neoplastic cells can appear which are profoundly resistant to amethopterin and in which the level of folic acid reductase activity is not significantly different from that in drug-sensitive cells. Accordingly, other mechanisms of resistance to amethopterin remain to be elucidated in murine, and probably in human, neoplastic cells.

Other factors may influence the incapacity of amethopterin to cure leukemia; for example, the question has been raised of the role of a virus supposedly responsible for the initiation of the leukemic state. If there is such a carcinogenic virus in human leukemic cells, is it destroyed when such cells die following exposure to amethopterin, or is the virus released to serve as a carcinogen elsewhere? Clearly, we have no definitive answer to this question as yet, but it does appear that, even if the neoplastic state has been induced by a virus, the latter, in many cases, is not retained by proliferating malignant cells. Another factor, and one of particular interest to the pharmacologist, is the capacity of leukemia cells to invade the central nervous system and to receive their nutriments from the cerebrospinal fluid (CSF), in an area of the body into which, unfortunately, this rather remarkable drug, amethopterin, is transported very poorly (85). Since the physicochemical principles involved in the transport of drugs across the so-called blood-brain barrier are now fairly well understood, it is not unlikely that the structure of amethopterin can be modified in such a way as to permit it to enter the CSF. Thus, leukemic cells, which otherwise would repro-

G. A. Fischer, personal communication.
duce in the nervous tissues with impunity and then re-enter the blood stream, might be effectively attacked within the brain as well as elsewhere.

5-Fluorouracil.—Another development of great importance in cancer chemotherapy has been the synthesis of 5-fluorouracil (5-FU) (26); of still greater significance to the concept of the metabolic approach are the preparation and study of its deoxyribonucleoside, 5-fluorodeoxyuridine (20, 27). Although several groups had appreciated the potential significance of blocking the position in the pyrimidine ring to which a methyl group must be attached in the formation of the thymine moiety of thymidylic acid, and compounds had been made in which the 5-position was blocked with nitro, amino, or other groups, or with the halogen atoms iodine, bromine, or chlorine, all attempts to introduce the fluorine atom into position 5 of uracil had failed until this was accomplished by Duschinsky, Pleven, and Heidelberger (26). This remarkable compound, 5-fluorouracil, and such congeners of it as 5-fluorocytosine, 5-fluoroacetic acid, 5-fluorouridine, and 5-fluorodeoxyuridine, have been studied extensively by the group at Wisconsin and by others, and the principal aspect of the mechanism of action of these compounds can now be explained at the enzyme level.

The most important of the several metabolites of 5-fluorouracil appears to be 5-fluorodeoxyuridylic acid (i.e., 5-fluoro-2′-deoxyuridine 5′-phosphate), a compound which can be formed more directly within cells by the administration of 5-fluorodeoxyuridine, often referred to as FUDR. In this metabolite, the relatively small fluorine atom (weight, 19; van der Waals radius, 1.35 Å) closely resembles hydrogen (weight, 1; radius, 1.2 Å) and is so rigidly bound to carbon-5 that it cannot be displaced enzymatically. FUDR, as the 5′-monophosphate, behaves very much like deoxyuridylic acid and exhibits a high affinity for the enzyme, thymidylic acid synthetase, thereby inhibiting the formation of thymidylic acid (7, 50). It will be recalled that this enzyme catalyzes the reaction between deoxyuridylic acid and N³,N⁴-methylene-tetrahydrofolic acid.

These two compounds, 5-fluorouracil and its very costly derivative, 5-fluorodeoxyuridine, often induce dramatic effects upon the growth of certain neoplasms, and marked regressions occasionally occur, particularly with carcinomas of the breast and lower bowel, but also with some other types of tumors. Unfortunately, however, the metabolic derivatives of these compounds exhibit such marked inhibitory activity upon this rather ubiquitous enzyme system, thymidylic acid synthetase, which ordinarily is essential for the reproductive activity of all cells, normal or malignant, that their activity as inhibitors of neoplastic growth usually can be exhibited only at the cost of pronounced effects upon normal cells. As a result, the manifestations of toxicity which the treated individual must experience limit greatly the usefulness of these chemotherapeutic agents (1, 23). Nevertheless, these compounds are dramatic examples of biochemical ingenuity and of the use of sound metabolic principles in the design of chemotherapeutic agents. Furthermore, 5-fluorodeoxyuridine, administered continuously into the blood stream, particularly into the arterial supply of accessible neoplasms, and especially in combination with other agents, appears to offer considerable promise and to justify much more investigation. More will be said about these areas in connection with discussions of 5-iodo- and 5-bromo-compounds, which act at near, but different, enzymatic sites and interfere with the formation of DNA, as well as lead to modifications in its structure.

The development of resistance to 5-fluorouracil by cancer cells grown in mice has been reported (51, 76), and neoplastic murine mast cells resistant to 5-fluorodeoxyuridine have been selected in culture (64). Although it appeared that such resistance is attributable mainly to defects in the capacity of the cells to phosphorylate uridine (and 5-fluorouridine) or deoxyuridine (and 5-fluorodeoxyuridine) (51, 64, 76), recent studies by Reichard and Sköld (75) have demonstrated that, in vivo, strains of Ehrlich ascites tumor cells may become quite resistant to 5-fluorouracil before decreases in uridine kinase activity occur. Thus, one or several unknown factors may contribute to early resistance to this drug.

6-Mercaptopurine.—Although there is insufficient opportunity in this article to present a detailed discussion of an important purine antimetabolite, 6-mercaptopurine (6-MP), mention should be made of it, because 6-MP not only is of great value in the treatment of certain leukemias, but it also represents an important development in the metabolic approach to cancer chemotherapy. Actually, 6-MP may be regarded as a product of what its discoverers have rather aptly referred to as “rational empiricism.” By this quaint phrase is meant the extension of biological observations with the aid of both metabolic logic and chemical empiricism. Thus, while studying the effect of a variety of compounds of related structure on the growth of folic acid-dependent microorganisms, it was observed that certain pyrimidine- and purine-derivatives profoundly inhibit...
growth (29, 54, 55). Of the various substituted purines, 6-MP was regarded as particularly promising. After many studies of its effects upon the growth of various transplanted tumors in mice, and of its chronic toxicity in higher animals, 6-MP eventually came to clinical trial. Despite the efforts of many biochemists, its mechanism of action still remains far from completely clarified (49); however, much of what is known is summarized in the following rather oversimplified discussion.

In a manner comparable to that by which such purines as hypoxanthine and guanine are used by cells, 6-MP first is converted to the corresponding ribonucleotide (8, 62). The structure of the product closely resembles that of the first compound to emerge in the biosynthesis of purines de novo—namely, hypoxanthine ribonucleotide or inosinic acid (11), differing from it only by the replacement of an oxygen atom at position-6 by one of sulfur. This metabolite of 6-MP appears to inhibit the conversion of inosinic acid to the essential compounds, adenylic acid and guanylic acid, as well as their interconversion (78). There is no convincing evidence that the very limited incorporation of the ribonucleotide of 6-MP into nucleic acids plays a significant role in the mechanism of action of the analog. On the other hand, a rather new development with respect to the mode of action of 6-MP is the effect of this abnormal compound on what have been termed “negative feedback mechanisms” (36-38, 96, 97).

The logic of using antimetabolites to mimic naturally occurring intermediates in such feedback mechanisms or as suppressors or repressors of enzyme syntheses has only recently come under consideration, but already it appears that this concept has considerable promise for the future of the metabolic approach to chemotherapy. Accordingly, it may be worth while briefly to discuss, albeit in a much over-simplified manner, the negative feedback principle of the control of a metabolic sequence of reactions. Let us consider a series of metabolic reactions in which compounds, as designated by letters, are modified: A → B → C → D → E. In such a series, it would not be surprising if, as a result of the inhibition of the conversion of D to E, with resultant accumulation of D, a product-inhibition of the conversion of C to D occurred. However, through negative feedback we must visualize, when adequate amounts of E are available, an inhibition of much earlier steps in the sequence of reactions—for instance, that which is concerned with the conversion of A to B. In only a few cases is the mechanism of such an inhibition even partially understood; however, it will be appreciated that such a controlling mechanism offers a means of regulating the overall sequence of reactions and of preventing the unnecessary accumulation of such compounds as B, C, D, and E, when adequate amounts of E are available. However, another mechanism of metabolic regulation—namely, repression of enzyme synthesis—must be considered in this and other situations.

An example of negative feedback in the nucleic acid metabolism of bacteria was provided by Yates and Pardee (97), who showed that the first step in the biosynthesis of pyrimidines de novo could be inhibited when uracil was added to a whole cell system or when cytidine 5'-phosphate was added to an appropriate cell-free extract. More pertinent to our present interest in 6-MP is the feedback inhibition of the de novo biosynthesis of purines, also at the first stage—namely, that of the formation of ribosylamine 5-phosphate (96). Thus, a recent report (61) that 6-MP and other purinethiols did not cause the accumulation of α-formylglycinamide ribonucleotide, a fairly early intermediate in the biosynthesis of purines, may have less pertinence than would a study of the effect of such agents on the first reaction—i.e., the formation of ribosylamine 5-phosphate.

Several years ago it had been observed that the administration of 6-MP causes a marked inhibition of the incorporation of formate-C14 into newly formed purines, a rather mysterious finding at the time (49, 61). Now, however, this result can be interpreted as signifying that the analog, presumably as its metabolically derived ribonucleotide, mimics the effect of such a naturally occurring purine ribonucleotide as inosinic acid and suppresses thereby a very early step in purine biosynthesis; this effect might be referred to as control by “pseudo-negative feedback.” Although it is not meant to imply that this is a definite mechanism of action of 6-MP on tumors, it may be a significant one, and the negative feedback principle should be given further consideration in attempting to explain the actions of this and other antimetabolites.

Before leaving the discussion of 6-MP, it is important to mention recent studies at the enzyme level which clarify at least one of the mechanisms by which resistance to this agent may be accomplished (2, 8, 9). In this situation, the drug leads to the death of those neoplastic cells which are able to convert it enzymatically into the ribonucleotide, while those cells which, presumably fortuitously, are deficient in the enzyme system that converts such free purines as hypoxanthine and guanine to inosinic acid and guanylic acid,
respectively, cannot convert 6-MP into the corresponding ribonucleotide, and, therefore, survive in the presence of the drug.

It would be appropriate at this point to mention the possibility that such drug-resistant cells, by virtue of their deficiency in functional enzyme for utilizing the above-mentioned pre-formed purines, might have been expected to have absolute dependence upon the de novo pathway for their supply of essential purine-containing compounds. If this were actually the case, however, it would be anticipated that such drug-resistant cells would be selectively susceptible to an inhibitor (e.g., amethopterin) of the de novo pathway. The results of such chemotherapeutic studies of 6-MP-resistant neoplasms have been disappointing and, at first glance, are seemingly inexplicable. However, it now appears that an answer to this seeming paradox has been obtained from additional enzymatic investigations. Thus, the deletion of the enzymatic capacity for the ribonucleotidation of hypoxanthine and guanine does not appear necessarily to include a loss of the capacity to convert adenine to adenylic acid, for which a different pyrophosphorylase is needed (9). Since adenylic acid can thus be formed and this can be converted to guanylic acid, the cells are put to no great trouble to acquire their essential purine derivatives.

Clearly, a metabolic implication of such findings is the need for a logical attempt to devise a selective inhibitor of the enzyme which converts adenine to adenylic acid; thus, in such 6-MP-resistant cells, a specific inhibition of this conversion, perhaps through the use of an analog of adenine, in combination with amethopterin, could be selectively lethal. Under such circumstances, it would be theoretically possible to protect the normal cells of the host, e.g., with hypoxanthine, together with thymidine to overcome the blockade in thymidylate acid synthesis imposed by the folic acid antagonist. There is need for vigorous investigation in this and similar areas of what has been termed conditioned selectivity (86), other potential examples of which will be presented.

Azauridine.—It is desirable to use a portion of the available space for a discussion of a recent development in the metabolic approach to cancer chemotherapy which was initiated in our laboratories at Yale. This investigation has been concerned with a new agent, 6-azauridine, the ribonucleoside of an analog of uracil, 6-azauracil (astraizine-3,5-[2H,4H]-dione). The latter was prepared in a deliberate attempt to obtain a specific antagonist of uracil (3, 30), since it had become apparent that certain neoplasms, in contrast to previously held concepts, are capable of using this free pyrimidine for the biosynthesis of nucleic acids (16, 68, 77).

Although 6-azauracil markedly inhibited the growth of a variety of microorganisms, an effect specifically antagonized by uracil, it soon became apparent that a much more subtle mechanism was involved than competitive antagonism of the utilization of uracil (48). Nevertheless, because it inhibited the growth of several mouse tumors, azauracil was subjected to studies of its chronic toxicity in dogs and monkeys (58, 87, 88). It was found that in divided doses supplying as much as 90, or even 180, mg/kg/day, the compound caused no toxic effects in the monkey, although its continued administration to puppies, in doses of about 60 mg/kg/day, led to loss of weight and disturbances of gastrointestinal function; however, it should be emphasized that in neither species was there any discernible manifestation of an effect on the central nervous system. Consequently, it was most surprising to find, in the first and in all subsequent patients studied, that the continued administration of azauracil led to unequivocal alterations in cerebral functions. These were characterized by drowsiness, dizziness, clonic movements of the extremities, marked changes in the electroencephalographic patterns, and even hallucinations. Although promptly reversible when the administration of azauracil was stopped, the disturbances were of such magnitude as to preclude, in most patients, the continued use of azauracil as a potential chemotherapeutic agent (87, 88, 93). It may be mentioned in passing that this serenditiously observed effect of azauracil on the nervous system of man, which rather clearly distinguishes the brain of Homo sapiens from that of the monkey, is deserving of much more intensive investigation than it has, as yet, received. As will be developed later, the mechanism of this unexpected effect of azauracil is quite unrelated to its action as a chemotherapeutic agent.

While these studies of 6-azauracil were in progress, work by Handschumacher in our laboratories had shown clearly that the active form of the inhibitor of cell growth, as is the case with several other agents, is the ribonucleotide, 6-azauridine 5'-phosphate or azauridylic acid. Furthermore, rather than functioning as a competitive antagonist of the apparently corresponding essential metabolite, i.e., uridine 5'-phosphate (UMP or uridylic acid), the site of action proved to be the enzyme responsible for the de novo formation of uridylic acid, via orotidylic acid (43, 47).

To understand this mechanism, it will be helpful to review briefly the pathway of de novo biosyn-
thesis of pyrimidines within cells (22). The non-
essential amino acid, aspartic acid, is converted
into N-carbamylaspartate (ureido-succinate); after
ring-closure to form dihydro- orotate and after
oxidation to orotate, ribonucleotide reaction occurs,
with the formation of orotidine 5'-phosphate (oro-
tidyllic acid). This compound is immediately at-
tacked by a specific enzyme, orotidyl acid de-
carboxylase; thus, with the loss of CO$_2$, uridylic
acid is formed. It is as a competitive inhibitor
of this decarboxylase that azauridine 5'-phosphate
acts, and there is, as yet, no convincing evidence
to indicate that the compound produces other
effects of significance to the functions of animal
cells.

Since mammalian cells are inefficient in convert-
ing azauracil to the functional ribonucleotide, and
because of the untoward effect of azauracil on
the nervous system of man, the ribonucleoside,
azauridine, was prepared. Although this was done
both chemically (44) and microbiologically (42),
it is with the aid of bacteria that this extremely
costly derivative has been prepared in large
amounts. For this purpose, $E.~coli$ is grown in
a salts-glucose medium and, at an appropriate
stage, 6-azauracil is added (88). Under these con-
ditions, the organism obligingly forms the desired
derivative and excretes it into the medium from
which it may be isolated.$^2$ As a manifestation of
its site of enzymic inhibition, there are also found
in the $E.~coli$ medium remarkably large amounts
of orotic acid and its ribonucleoside, orotidine,
as well as variable amounts of orotidyl acid and
uridylic acid (43).

Azauridine is now being investigated clinically
in several medical centers, particularly at Yale,
the National Cancer Institute, and the Roswell
Park Memorial Institute in this country, and by
a group in Czechoslovakia (87). The compound
is remarkable for its chemical and biological sta-
tility; thus, unlike the ribonucleosides of various
naturally occurring bases, as well as those of many
other analogs, azauridine apparently is not cleaved
to any significant degree by mammalian nucleo-
sidase systems. In fact, the majority of parenteral-
ly administered azauridine is rapidly excreted in
the urine, from which much of it can be recovered
unchanged.

$^2$6-Azauridine was first provided to the group at Yale
through the generosity of the Squibb Institute for Medical Re-
search, and later by the Cancer Chemotherapy National Serv-
vice Center (C.C.N.S.C.) of the Public Health Service, under
contract with either the E. R. Squibb Division of the Olin-
Mathison Co. or with the California Corp. for Biochemical Re-
search; the C.C.N.S.C. has also provided the compound for
clinical investigation by other groups, particularly those at the
National Cancer Institute (and associated hospitals) and the
Roswell Park Memorial Institute (Buffalo, N.Y.).

In normal dogs, azauridine, which is well ab-
sorbed from the intestine of this species, caused
anorexia and profoundly inhibited the formation
of leukocytes, not only of granulocytes, but also
of lymphocytes. Continued administration of the
compound, 18–27 mg/kg, in three divided
oral or parenteral doses daily, led to a fatal
result within 7–10 days, unless administration
of the compound was stopped and supportive
therapy was given.

Accordingly, in man azauridine was adminis-
tered very cautiously, at first by mouth and later,
when it was found not to be well absorbed from
the human intestine, by the intravenous route.
Even then, however, no toxic effects were pro-
duced, although the compound has been given
during periods of many days in doses 10 or 20
times those which were lethal in the dog. It is
important to emphasize that these huge intrave-
nous doses of the ribonucleoside did not cause any
manifestations of central nervous system toxicity,
in contrast to the results obtained with the free
base, azauracil (87, 88). It is now clear that this
effect of azauracil on the function of the human
brain is unrelated to the inhibition of orotidyl
acid decarboxylase caused by azauridine 5'-phos-
phate (18, 87).

The very poor absorption of azauridine from
the gastrointestinal tract of man, coupled with the
exceedingly rapid renal excretion of this com-
 pound, has necessitated, until recently, the ad-
mnistration of azauridine intravenously, either by
continuous infusion or at 8-hour intervals. This
problem has now been circumvented, however, by
the preparation of $2',3',5'$-triacetyl 6-azauridine, a
lipide-soluble compound which, given orally at
intervals of 8 hours, is completely absorbed by
man. Following the oral administration of this
derivative, sustained blood levels of free azauri-
dine are achieved; of the material excreted in the
urine, approximately 80 per cent is free azauridine,
while about 17 per cent is the 5'-mono-acetyl
derivative (only traces of triacetyl azauridine are
excreted). In leukemia patients, preliminary
studies have shown that orally administered tri-
acetyl azauridine causes the same metabolic
blockade and clinical effects as those produced by
molar equivalent amounts of azauridine given
intravenously. It is suggested that the new deriva-
tive of azauridine will be the compound of choice,
except in those individuals in whom oral therapy
is precluded (46).

The remarkable species difference between man
and dog, with respect to the effect of azauridine
on the formation of leukocytes, has now been
partially clarified. It appears that in the canine
cells there is a profound dependence on the de
...novo-pathway for a source of pyrimidines, whereas in normal man it seems likely that this pathway has but little importance. It is possible, therefore, that many other normal cells also do not depend primarily on orotic acid as a source of their pyrimidines, since the continued administration of massive doses of azauridine has caused no evidence of deleterious effects; however, it remains to be determined to what degree azauridine is converted to the functional 5'-phosphate by various human cells, other than leukocytes (17).

In the light of this situation, it has been interesting to find that, in some cases of human leukemia, particularly acute monocytic, but also more typical forms of acute granulocytic and acute phases of chronic myelogenous leukemia, rather striking, albeit temporary, partial remissions may occur following the frequent intravenous administration of azauridine, again without the detection of undesirable side-effects. As indicated earlier, these results seem to place azauridine in a unique position among cancer chemotherapeutic agents, since for the first time lethal effects upon a malignant cell-type can be obtained without analogous effects on normal reproducing cells.

As a tentative explanation of these observations, it may be suggested that in certain leukemic cells of man there is a biochemical defect in the utilization of preformed pyrimidines; as a result, the cells are forced to obtain their complement of uridine from the de novo-pathway. Since it is this latter pathway which azauridine 5'-phosphate inhibits, the neoplastic cells are left without an adequate complement of pyrimidines, and, as a result, cell death occurs. It is important to point out, however, that, for such cells to be sensitive to azauridine, they cannot have lost completely the capacity to phosphorylate the normal metabolite, uridine, since the enzyme responsible for this phosphorylation, uridine kinase, appears to be responsible for the conversion of azauridine to its 5'-phosphate, the active inhibitor of orotidyl acid decarboxylase. The amount of uridine kinase activity in certain malignant leukocytes of man is probably inadequate to supply their needs for uridine 5'-phosphate from the small amounts of uridine present in the blood and tissue fluids, and only with massive doses of azauridine is a sufficient amount of azauridine 5'-phosphate formed within the cells that the desired degree of inhibition of orotidyl acid decarboxylase occurs.

It is important to note that the azauridine-produced inhibition of the de novo-pathway of pyrimidine biosynthesis in malignant leukocytes gradually disappears, despite the continued administration of the drug. This finding may reflect, in part, the gradual accumulation of orotidyl acid. It will be recalled that there is a competitive relationship between orotidyl acid and azauridyl acid (one molecule of the latter is overcome by about ten molecules of orotidyl acid [49]). In addition, there is preliminary evidence to suggest that an adaptive synthesis of orotidyl acid decarboxylase gradually occurs under these conditions. Indeed, within a few days following the withdrawal of azauridine therapy, with a resultant diminution in the levels of azauridine 5'-phosphate and orotidyl acid within the cells, the reproduction of leukemic cells may be restored and the subsequent administration of azauridine again may induce a partial remission (87).

These findings point the way to a logical metabolic approach to better chemotherapeutic results with azauridine, for, if there were a way to suppress the accumulation of orotidyl acid within azauridine-treated cells, the tendency for such metabolic reversal might be greatly diminished. Reflection upon the chemical structure of the intermediates involved in the pathway of biosynthesis de novo of pyrimidines will suggest a variety of logical approaches to the design of inhibitors of orotidylate synthesis, and investigation in this area is actively in progress.

There is another important reason why a potent inhibitor of the de novo pathway of pyrimidine biosynthesis could have useful chemotherapeutic applications. This concept is an outgrowth of precise information concerning an enzymatic basis for resistance to azauridine. Thus, studies with mouse leukemia cells have shown that the cells selected by this drug, which become the progenitors of an azauridine-resistant population, are those that are markedly deficient in uridine kinase activity (67). Such cells, which cannot phosphorylate azauridine effectively, for the same reason may be denied the use of preformed uridine; consequently, such cells should be unusually sensitive to an inhibitor of the de novo-pathway of formation of uridyl acid. Thus, the design of an effective inhibitor in this sequence of reactions could permit chemotherapeutic exploitation of such a metabolically established instance of "conditioned selectivity" (86). It could be anticipated that the cells of normal human tissues, having an ample supply of uridine kinase, might not be influenced by such an agent, since their complement of uridyl acid presumably could be obtained from extracellular sources of uridine.

Halogenated pyrimidine deoxyribonucleosides (other than FUDR).—In the space which remains, it would be desirable to review briefly another...
metabolic approach to cancer chemotherapy which is being actively pursued in other laboratories as well as ours. These investigations are concerned with compounds which either interfere specifically with the incorporation of thymidylic acid into DNA or are able, to some extent, to substitute for thymidylic acid in the biosynthesis of DNA, and thus to lead to the formation of an abnormally constituted macromolecule.

The theoretical basis for the design of antimetabolites specifically constructed to interfere with the biosynthesis of DNA will need no great elaboration here. At this time, almost all biological scientists have become generally familiar with the recent great advances in knowledge which bears on the structure, biosynthesis, and function of deoxyribonucleic acids. Since a cell cannot undergo mitotic cleavage unless it first has replicated its gene-components, and since these are composed primarily of deoxyribonucleoproteins, it follows that a significant interference with any of the reactions essential to the synthesis of DNA could profoundly diminish the reproductive capacity of cells. Although doubtless many approaches are possible, a most promising one has seemed to be to interfere with the formation or utilization of the component of DNA which contains the unique base, thymine. As has been described already, both amethopterin and 5-fluorodeoxyuridylic acid, by different mechanisms, profoundly inhibit the formation of thymidine 5'-phosphate; but neither compound significantly influences the utilization of this thymine derivative, once it has been formed.

Work along these lines began several years ago with studies by Prusoff and the writer, and their associates, of 6-azathymine (6-methyl-astro-triazine-3,5-[2H,4H]-dione), and its deoxyribonucleoside, azathymidine (71, 73, 92); although the latter compound is a weak antagonist of thymidine, it has offered no promise of usefulness in vivo. Earlier attempts to obtain antimetabolites in this area also included studies of the 5-iodo derivatives of orotic acid, uracil and uridine (72); then, when 2'-deoxyuridine became available, 5-iodo-2'-deoxyuridine (IUDR) was prepared by Prusoff (70).

Iodine substitution was chosen primarily because of the close similarity between the van der Waals radius of this atom and that of the methyl group in thymidine (1, 2.15 A and CH3, 2.0 A). Although an equally good or possibly better case could be made for substitution with bromine (radius, 1.95 A), preliminary studies have indicated that, under conditions encountered in vivo, e.g., in the inhibition of tumor growth in mice, 5-bromo-2'-deoxyuridine is much less active than is IUDR.

To summarize a great deal of investigation with IUDR (15, 91), the compound proved (a) to be an antagonist of the incorporation of thymidine into the DNA of mammalian cells, (b) to be incorporated, as a deoxyribonucleotide, into DNA, (c) to inhibit the growth of several transplanted neoplasms in mice, (d) gradually to produce leukopenia and thrombocytopenia when injected repeatedly into dogs, (e) occasionally to cause regressions of solid tumors in man, and (f) to potentiate the effects of subsequent exposure of tumors to x-radiation. Favorable effects on human neoplasms, with the regimens of dosage used until recently, have been seen occasionally, but only after a maximally tolerated amount of IUDR had been given during a period of 5 or 6 days. To attain these effects, this rather poorly soluble and chemically and biologically unstable compound was administered by intravenous infusion during 2-hour periods, in dosages of 100-120 mg/kg daily, usually for 5 successive days.4

It is notable that such effects could be obtained when IUDR was administered by this technic, since about 15-20 per cent of the injected compound is rapidly excreted unchanged by the kidneys and most of the remainder of the injected material is rather rapidly catabolized to 5-iodouracil and to iodide. Thus, studies in man with IUDR-containing radioactive iodine have shown that only during the 2-hour intravenous infusion and for about 2 hours thereafter were significant concentrations of IUDR present in the blood, as reflected by the urinary excretion of the intact substance (about 13 per cent was excreted during a 4-hour period which includes the time required for intravenous infusion). So rapidly was the compound catabolized that, within the same 4-hour period, another 20 per cent of the radioactive iodine was excreted in the urine as 5-iodouracil and iodide; and, during the subsequent 6 hours, another 25 per cent was excreted as these degradation products. In fact, within 24 hours about 88 per cent and within 48 hours about 94 per cent of the total administered radioactivity was excreted in the urine. Nevertheless, such a regimen of five intermittent dosages at daily intervals

4 5-Iodo-2'-deoxyuridine, in the relatively large amounts required for the various investigations so far carried out at Yale, has been provided either by the Cancer Chemotherapy National Service Center (C.C.N.S.C.) of the Public Health Service or with funds generously supplied by the Connecticut Division of the American Cancer Society; the C.C.N.S.C. has also provided the compound for clinical investigation by other groups, particularly those at the Sloan-Kettering Institute and the Universities of Wisconsin and California.
may produce a variety of unpleasant effects on normal tissues; the most hazardous of these is leukopenia, and a very uncomfortable one is severe stomatitis; but that which is of least consequence, although often particularly disturbing to the patient, is alopecia. It is worth noting, however, that no definite evidence of intestinal toxicity has been seen.

That some of these undesirable effects in man can be prevented by the appropriate use of the corresponding metabolite, thymidine, has been convincingly demonstrated by Calabresi (13). In these studies a small amount of thymidine (4 mg/kg) was infused into one external carotid artery during a period of about 4.25 hours, initiated about 1/4 hour prior to the 2-hour intravenous infusion of IUDR (120 mg/kg). Carried out for 5 successive days, in four patients in whom the effects of IUDR alone had been assessed previously, no interference with the effect of the drug on leukocyte formation occurred, but stomatitis was prevented completely, and a marked reduction in the loss of hair occurred on the infused side of the cranium. Subsequently, studies by Calabresi (14), using similar infusions of small amounts (8 mg/kg) of thymidine into a hypogastric artery, apparently permitted a sufficiently large amount of bone marrow to escape the effects of IUDR as to reduce significantly the magnitude of the leukopenia which ordinarily follows the usual regimens of intravenously administered IUDR. Since this procedure has not protected the oral mucosa and hair follicles from the effects of the drug, it seems possible that, with even higher intravenous dosages of IUDR, much more profound effects upon certain types of neoplasms should now be attainable without the hazard of hypoplasia of the bone marrow.

Prusoff and his associates have recently obtained evidence that IUDR (or, more likely, its phosphorylated derivatives) exerts inhibitory effects at various reaction sites, the primary one being related to the nature of the DNA precursor involved, as well as the variety of cell studied. Thus, DNA-polymerase was primarily inhibited in mouse Ehrlich carcinoma cells and in the leukocytes of patients with acute monocytic or chronic granulocytic leukemia; on the other hand, with cells of a murine leukemia strain (L5178Y), thymidine kinase was preferentially inhibited with thymidine-5'-H as the precursor, while thymidylic acid kinase was the site of primary inhibition when formate-C\(^14\) was the labeled precursor of DNA-thymine.\(^5\)

That IUDR is converted to the triphosphate in cells had been clearly indicated previously by its rapid appearance in DNA in place of an equivalent amount of thymidylic acid (28, 63, 84). Thus, in a growing culture of mouse leukemia cells, a relatively low concentration of IUDR (50 \(\mu\)g) permitted the cells to undergo only one cell division; under these conditions, about one-third of the thymidylic acid of the DNA was replaced by 5-iodo-2'-deoxyuridylic acid. Since only one cell division took place, it can be deduced that, in the new DNA laid down before mitosis, about two-thirds of the thymidylic acid in a single strand was replaced by the analog; this level of incorporation appeared to be lethal (63).

Although only very preliminary studies have been made of the extent of uptake of IUDR into the DNA of human tumors, it appears that, under the conditions of dosage which have been studied so far, the amount of incorporation of the analog into metastatic tumor tissue in situ has been very small. Perhaps it is not surprising that this should be the case, since, even under conditions of exponential reproduction of most mammalian cells in culture, not more than about a third of the cells, at any given time, are synthesizing DNA, and, under the conditions which obtain in most human neoplasms in situ, a very much smaller proportion of the cells probably is forming DNA during such a relatively brief period of exposure to IUDR as 4 hours.

At this stage of the investigations it is not possible to conclude that high levels of incorporation of IUDR into DNA are an essential feature of the carcinolytic or carcinostatic effects of this agent. If such incorporation proves to be desirable, very long-continued exposure of tumor cells to IUDR will be indicated; however, the technical complications of such a procedure, involving perhaps the introduction of the drug at a constant rate into the arterial supply of a neoplasm, appear to be rather baffling. Under the conditions of present knowledge, such prolonged procedures would be neither safe nor feasible; accordingly, intravenous infusion of the agent for many days may be indicated, with a view to affording the tumor cells a maintained concentration which should favor incorporation of the drug into DNA. There is an additional motivation for attaining the formation of "fraudulent DNA," namely, to reduce the threshold of the cells to radiation injury, about which it is now appropriate to comment.

Studies with a thymine-deficient strain of \(E.\)\( coli\) by Greer (40) demonstrated a marked increase in sensitivity to ultraviolet radiation after growth

in the presence of 5-bromouracil, a circumstance which led to a considerable replacement of the thymine of the DNA with the analog. Also, Szybalski and his associates (25, 84) have shown that either 5-chloro- or 5-bromo-2'-deoxyuridine and, to a lesser extent, IUDR, can be incorporated extensively into the cellular DNA of human cells grown in culture. With the chlorine and bromine compounds, replacement of 50-60 per cent of the DNA thymidine could be attained without loss of viability, if accomplished gradually during many successive reproductive cycles. Usually such incorporation was facilitated by profoundly inhibiting the biosynthesis of thymidylic acid through addition to the medium of 5-fluoro-2'-deoxyuridine (FUDR), under which conditions the reproduction of cells then was greatly stimulated by the further addition of 5-bromo-2'-deoxyuridine (BUDR), with much better incorporation of the bromo compound into DNA than occurred in the absence of FUDR. With continued replications of DNA in the presence of these analogs, a bromine atom replaced the methyl group of a large proportion of the thymidylic acid components of both strands of the double helix of DNA. Such dual replacement was believed to favor a very definite reduction in the threshold of such cells to the effects of exposure to either ultraviolet-or x-radiation. Indeed, it was suggested that the halogen-containing compound must be present in both strands of the DNA, since, with continued replication of these mammalian cells in the presence of the analog, sensitivity to radiation was retained, whereas, with a single subsequent replication in the absence of the analog, restoration of relative resistance to radiation-injury was reported (25). On the other hand, as has been suggested by Kligerman, the possibility that “sensitization” might be dependent upon the presence of phosphorylated derivatives of the analog in the cytoplasm, rather than in DNA, did not appear to have been precluded, since it is reasonable to expect that a marked diminution in the amount of these compounds in this fraction of the cells would occur during one reproductive cycle in the absence of the analog.

Recent studies by Kaplan and associates (59) with strains of E. coli grown in the presence of halogenated pyrimidines have indicated that the radiosensitization conferred by these compounds is indeed dependent upon their incorporation into DNA, although a definitive explanation of the mechanism responsible for this result could not be offered. It was suggested, however, that labeling of only one strand of the double helix of the DNA was sufficient to confer at least half-maximal radiosensitization. These workers have appropriately pointed out the importance of this observation, for “similar” labeling of a high proportion of the cells of human neoplasms “would require that tumors be permitted to double in size between three and four times in the sustained presence of the analog before appreciable enhancement of radiosensitivity could be expected.” Accordingly, the possibility was suggested that “alternate use of the analogs and irradiation, if spaced at optimal intervals, could label and then selectively destroy a small proportion of the cells at each cycle, leaving the more radioresistant cells behind to be labeled in a subsequent cycle. If such an approach enabled complete sterilization of the tumor with as little as a 10 per cent reduction in total dose, it would represent a very real advance, since the margin of safety in many radiotherapeutic situations is small” (59).

Although it is evident that definitive explanations of the remarkable effects of these halogen-containing precursors of DNA on the threshold to radiation injury are not yet possible, various hypotheses are currently under examination. Certainly, from the standpoint of practical chemotherapy there is urgent need to determine what techniques of administration of compounds that cause radiosensitization are most nearly optimal, not only to facilitate incorporation of the appropriate analog into DNA but also to concentrate the effects of such compounds on the neoplastic cells and to minimize their effects on the actively reproducing normal cells of the body. Since data obtained from cell culture indicate that, for significant effects on cell growth, concentrations in excess of 2 μg of IUDR/ml must be maintained in the extra-cellular fluid, it will be appreciated that the therapeutic problems involved are indeed perplexing.

A possibly encouraging note comes, however, from the development of a derivative of BUDR—namely, 5-bromo-2'-deoxycytidine (BCDR) (19). It had been shown that cytosine-containing nucleosides, unlike those of uracil or thymine, do not participate in certain enzyme-catalyzed reactions which lead to a displacement of their sugar components (24). This resistance to catabolic influences on the part of normal metabolites suggested that BCDR and 5-iodo-2'-deoxycytidine (ICDR) might have the metabolic stability which is so obviously lacking with IUDR. This “metabolic approach,” which led first to the development of an improved synthesis of the bromine derivative (19), appeared to have been justifiable, because not only is BCDR resistant to nucleosidases (21),

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*M. M. Kligerman, personal communication.*
but also, in mice, bromide appears to be cleaved from it less rapidly and less extensively than it is from BUDR (60). As expected, BCDR is converted enzymatically into intracellular 5-bromo-2'-deoxyuridyllic acid; but whether this is accomplished by deamination subsequent to phosphorylation of BCDR, or whether deamination also occurs before phosphorylation, is not yet certain. In any case, the compound which appears in the DNA of cells grown in the presence of BCDR is 5-bromo-2'-deoxyuridine 5'-phosphate (21). Despite these encouraging findings, BCDR-Br\(^{18}\) appears to be as rapidly debrominated by man, following its intravenous administration, as is BUDR. However, several features concerning the effects of BCDR on cancer cells, as compared with those of BUDR, have indicated that additional studies of BCDR should be carried out, and these are now in progress in several laboratories. Even more interesting will be studies of 5-ido-2'-deoxyxycytidine (ICDR), a compound which proved to be unexpectedly difficult to synthesize, although this has now been accomplished.\(^8\)

In conclusion, many metabolic challenges remain for additional investigation. There are numerous opportunities for the further exploitation of conditioned selectivity (86)—e.g., the discovery of potent inhibitors of the utilization of adenine, which, together with amethopterin, could be of value in the treatment of tumors that are resistant to 6-MP because of their defective capacity to form the ribonucleotides of hypoxanthine and guanine. Similarly, a new metabolic goal is the development of effective inhibitors of the biosynthesis of orotic acid, or of its utilization, not only as potential synergists of azauridine, but also as possibly selective inhibitors of those neoplastic cells which are resistant to azauridine because of defective uridine kinase activity, with a resultant inability to obtain an adequate complement of uridine 5'-phosphate from exogenous sources of uridine. Also, there is the directed search for more useful compounds which act by suppressing the formation of DNA, particularly compounds that exhibit greater resistance to metabolic degradation by normal tissues, while possessing the necessary feature of utilization by neoplastic cells. And, of special interest is the development of better techniques for using such compounds for selective localization of their attack upon neoplastic cells, either through restrictions upon the areas of the body which they reach following their introduction or by the appropriate protection with their metabolic antagonists of such critical normal tissues as the bone marrow. A corollary of these metabolic approaches is the attempt to obtain significant and practicable reductions in the threshold of cells to radiation injury, a feature which appears to be dependent upon the formation of significant amounts of "fraudulent DNA."

Particularly encouraging, in the metabolic approach to cancer chemotherapy, is the finding that azauridine, an agent which is selectively toxic for some types of human neoplastic cells, is essentially devoid of toxicity for any normal human cell. This observation should serve to stimulate further searches for exploitable metabolic differences between normal and neoplastic cells and to dispel somewhat the aura of pessimism which has seemed to prevail in this area.

But even if none of these or other metabolic approaches should take us to the urgently desired goal, it is certain that, through continued metabolic investigation, important contributions will be made to our knowledge of the fundamental processes of cells, both normal and neoplastic, and especially to the biology and biochemistry of cell growth and reproduction and, perhaps, to the relation of viral proliferation to neoplastic change. Accordingly, we can be confident that valuable progress is being made toward the acquisition of that degree of understanding of the basic mechanisms of living material which is essential if real success in cancer chemotherapy eventually is to be attained.

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Vol. 21, December 1961


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1489


Some Metabolic Approaches to Cancer Chemotherapy

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