Some Pharmacologic Considerations Concerning the Control of Acute Leukemia

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SUMMARY

Studies on the mode of action of the presently useful anti-leukemic drugs describe the metabolic features of leukemic lymphoblasts which serve as a basis for killing these cells selectively. Individually, these agents are inadequate in most cases to maintain prolonged remissions. The combined use, however, of drugs which act differently has the potentiality of greatly increasing the selectivity of treatments and minimizing the development of drug resistance. In anticipation of the discovery of more effective drugs which may displace these, or the utilization of new information about regulatory macromolecules, amplification of the effectiveness of the presently useful agents can be attempted, in the manner discussed, with the information derived from studies of a physiologic regulator (cortisol), a pseudo-feedback inhibitor (6-mercaptopurine), and a vitamin antagonist (amethopterin).

Instead of reviewing the latest information on the pharmacology of anti-leukemic agents now being evaluated, it seems to be more appropriate to discuss the most widely used anti-leukemic drugs from the viewpoint of what we have learned and what we do not yet know about the basis for their selective action. If, among the first 100,000 compounds tested, any drug capable of curing acute leukemia had been discovered, it would not be an urgent matter to understand the anti-leukemic action of amethopterin, 6-MP, or corticosteroids. The mechanism of action of these and other palliative agents would be of secondary interest. In the absence of any curative agent, however, means of amplifying the effectiveness of the useful anti-leukemic drugs assume major importance. Indeed, several-fold increases in the duration of remissions presently induced could result in increased survival measurable in years rather than months. Knowledge gained gradually by this strategy may lead eventually to curative treatment. Thus, while the search for new drugs continues, recognition of the pharmacologic gaps in understanding the selective action of these anti-leukemic drugs can define areas deserving of more intensive study.

Correlation of In Vitro and In Vivo Data on Mechanism of Action

Although much has been said concerning the defects and difficulties in correlating chemotherapeutic data derived from experiments in mouse and man, not enough has been said about the correlation of in vitro and in vivo experiments using the same type of cells. The design of our experiments is influenced by the nature of the question to be answered. If we ask which enzymes are affected by anti-leukemic drugs, then some accomplishment can be recognized in the biochemical data bearing on this question. If we ask which of the enzymes affected by these drugs mediate the inhibition of growth of leukemic cells, then in most cases the available data are inadequate to provide a satisfactory answer. If we ask why corticosteroids are useful in the control of acute lymphocytic leukemia but affect the acute myelogenous type adversely, or why amethopterin acts on acute but not chronic lymphocytic leukemia, then we recognize a void characterized by speculation unrestrained by relevant data. There is a logical, indeed necessary, sequence of questions to be answered in attempting to explain the anti-leukemic action of these drugs. When these can be answered, we will know what limits the range of effectiveness of these drugs. Certainly, these and other useful agents do not lack potency. They lack selectivity.

Lack of correlation between enzyme inhibition in cell-free preparations and in intact cells need not be surprising in view of the different levels of complexity of the systems. Whereas the concentrations of drug, enzyme, and substrate can be controlled in cell-free systems, any experiments with intact cells involve kinetics which are complex because of the different rates of several reactions or functions. Even the simplest cell culture system involving constant concentrations of a drug (Dc) which is not metabolized requires definition of the rates of drug entry and drug exit, as well as the rates of association with and dissociation from the sensitive enzyme which is being

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2 The abbreviations used are: 6-MP, 6-mercaptopurine; RNA, ribonucleic acid; AIC, 4-amino-5-imidazolecarboxamide; DNA, deoxyribonucleic acid; FUDR, 5-fluorodeoxyuridine.
Inactivation requires activation in the cell and is exposed to enzymes capable of forming inactive products, then the contribution of each variable is much more difficult to define (Chart 1, Model I). When variations in the extracellular concentration of drugs, owing to distribution in body compartments, inactivation in the liver, and excretion by the kidney, are superimposed on such relationships, then the additional complexity of the situation can be appreciated, even if not dealt with. Yet the pattern of these interrelationships, which undoubtedly varies in different types of cells, determines the selective toxicity of these drugs for certain tissues or certain types of leukocytes. Obviously, the identification of those enzymes which are most critically related to the impairment of growth and the elucidation of the drug-enzyme relationships are first steps toward the analysis of more complex situations peculiar to living cells. The oversimplified relationships indicated in Chart 1 are relevant to instances in which interference with inactivation of 6-MP or modification of cellular transport of amethopterin alters the selective action of these agents in vivo. Despite considerable progress in defining the specificity of transport systems for amino acids and other classes of metabolites (12, 77), little has yet been done with combinations of drugs, one of which is intended to alter the cellular capacity for the influx or efflux of another drug.

**Metabolic Effects of Corticosteroids**

At present, it is not feasible even to diagram a cellular receptor for the corticosteroids. There is no evidence that cortisol combines directly with any enzyme, although this possibility certainly has not been excluded. As in the case of other hormones, expression of the metabolic effects of cortisol requires the functioning intact cell. The diverse changes in carbohydrate and protein metabolism which occur in vivo as a consequence of corticosteroid treatment obscure identification of the primary events associated with attachment of the hormone to receptors. There is remarkably little information concerning the affinity of glucocorticoids for cellular structures. Based upon physical properties, Munck (55) has directed attention to the ability of various steroids to concentrate at interfaces, and Willmer (79) has developed the concept that steroids might alter the physical properties of membranes by their insertion into the phospholipid-cholesterol layers. Although the occurrence of steroid-specific receptors must be assumed to satisfy pharmacologic concepts, the identification of these is lacking.

Changes in capacity for protein synthesis appear to be a major consequence of corticosteroid treatment. In this regard it may be noted that the response of target tissues to estrogens (53), androgens (47), and thyroxine (74) also involves synthesis of new protein as is indicated by the inhibition of their metabolic effects by puromycin. The induction of hepatic enzymes by the glucocorticoids provides a means of probing the underlying mechanism by which cortisol accelerates the selective synthesis of certain enzymes. Also, the role of these enzymes can be studied in relation to accelerated gluconeogenesis and amino acid catabolism. The rapidly increasing literature on this subject has been reviewed recently (65, 73).

Biochemical studies of cortisol-responsive enzymes in liver are numerous in contrast to comparable studies of cortisol-responsive peripheral tissues. Doses of cortisol which stimulate protein anabolism in the liver cause dissolution of lymphoid tissues. Whereas the glucocorticoids increase the hepatic capture of amino acids (58), their effect on muscle is to decrease accumulation of amino acids and histamine (80, 81). Substantial evidence now indicates that one of the early effects of corticosteroid treatment is an accelerated incorporation of precursors into RNA in liver (18, 29, 41, 43). Similar experiments with a cortisol-sensitive lymphosarcoma (P1798) indicate definite inhibition of the incorporation of glycine-14C into RNA within 6 hr following a single dose of cortisol (M. J. Pine, unpublished data). It is an attractive hypothesis that the formation of messenger RNA can be affected by cortisol, but the induction of only certain enzymes and the selective effect on lymphoid tissues would still require an explanation.

Whether it combines with membranal receptors or with certain species of nucleic acids, one action of cortisol is to increase the amounts of many different hepatic enzymes. It is noteworthy that similar changes in activity also occur in response to those physiologic conditions which induce a negative nitrogen balance or to feeding diets high in protein (65). In our studies of inducible enzymes in thymus and cortisol-responsive tumors (Walker 256 and Lymphosarcoma P 1798), alanine transaminase is markedly increased in activity in these tissues following administration of cortisol (56, 61, 62). This might be attributed
to concentration of this enzyme during tumor regression or thymic dissolution were it not for the lack of similar changes in aspartate transaminase in the same tissue samples. Significant inhibition of the Walker tumor occurs when diets containing 75% protein are fed, and, under these conditions, the activity of alanine transaminase increases several-fold. Much remains to be done to determine the extent to which the intentional alteration of protein metabolism may amplify the effects of corticosteroid administration.

**SITE OF ACTION OF 6-MERCAPTOPURINE IN VIVO**

Biochemical studies on the inhibition by 6-MP of purine biosynthesis and nucleotide interconversions have been reviewed thoroughly (9, 10, 50). Yet this area of investigation still serves to illustrate the need for greater effort to relate the evidence for a biochemical locus of inhibition in vitro to the question of which enzymatic reaction limits cellular growth. Recent data are relevant to this question.

The growth of cells in culture can be made completely dependent upon preformed purines added to the medium when amethopterin is used to block purine synthesis de novo. Under these conditions the site inhibited by 6-MP which causes inhibition of growth is apparently the enzymatic conversion of inosinate to adenylate (34). Indeed, a competitive relationship was found to exist between 6-MP and hypoxanthine or its derivatives; in the presence of adenine or its derivatives the relationship was noncompetitive in experiments with HeLa cells. However, when the growth of these cultures was dependent upon the biosynthesis of purines de novo, then, as is shown in Table 1, growth was 50-100 times more sensitive to 6-MP (35).

One explanation for this difference in sensitivity could be that the amount of inosinate synthesized de novo which is present in a cell at any 1 time is very much smaller than the amount derived from hypoxanthine or its derivatives supplied in the medium. This explanation seems unlikely, however, since the cells grow at the same rate under each of these conditions and the intracellular amounts of inosinate would be expected to be of the same order of magnitude. It seemed more reasonable to assume that another reaction, prior to the formation of inosinate, would be more sensitive to inhibition by the ribonucleotide of 6-MP when purines are synthesized de novo.

Data presented recently by Bennett et al. (4) showed that, when 6-MP and labeled compounds were administered intraperitoneally to mice bearing ascites tumors, the conversion of AIC to purines was not inhibited by amounts of 6-MP 8-20 times those that produced 50% or greater inhibition of synthesis de novo, measured by the incorporation of glycine and formate. This evidence clearly suggests that the primary site of action of 6-MP in vivo is prior to the formation of the ribotide of AIC. These experiments, however, were not designed to establish whether inhibition at this site actually causes impairment of the growth of cells.

When cells are grown under conditions in which purines are synthesized de novo, AIC can effectively prevent the inhibition of growth of S-180 cells (subline AH/S) caused by 6-MP (Chart 2) (35). High concentrations of 6-MP (above 0.1 mM) cause some inhibition even in the presence of relatively high concentrations of AIC. Such dependence upon concentration would not be observed if the early step in purine biosynthesis were the only site of inhibition by 6-MP. Typical product reversal would then be expected to occur. Thus, it appears that inhibition of the step between inosinate and adenylate occurs when the concentration of 6-MP is raised high enough to compete with the inosinate which is formed from the AIC supplied in the medium. Although 6-MP can inhibit growth in the presence of hypoxanthine or its derivatives, the amounts of these compounds supplied in the medium are unlikely to occur in vivo. Also, if adenine or its derivatives were available in vivo, then 6-MP would not be expected to have any effect on the growth of cells. The evidence presented by Bennett et al. (4) indicates that the de novo pathway for the formation of purines predominates in vivo. Our data support the interpretation that the major effect of the ribonucleotide of 6-MP which causes the death of cells is on an early step in the biosynthesis of purines.

**TABLE 1**

**DEPENDENCE OF THE GROWTH-INHIBITORY POTENCY OF 6-MERCAPTOPURINE ON THE COMPOSITION OF THE MEDIUM**

<table>
<thead>
<tr>
<th>Growth dependent on</th>
<th>6-MERCAPTOPURINE (50% INHIBITORY CONCENTRATION)</th>
<th>9-MERCAPTOPURINE (90% INHIBITORY CONCENTRATION)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Purine synthesis de novo</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Preformed purines</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Hypoxanthine or its derivatives</td>
<td>150</td>
<td>200</td>
</tr>
<tr>
<td>Adenine or its derivatives</td>
<td>&gt;1000</td>
<td>&gt;1000</td>
</tr>
</tbody>
</table>

a Eagle's medium.

b Determined in amethopterin (1 mM) medium supplemented with thymidine (30 µM), glycine (100 µM), and the corresponding purine, 100 µM for S-180 and 30 µM for HeLa cells.

**CHART 2.—Effect of varied concentrations of 6-mercaptopurine on the growth of Sarcoma 180 cells (AH/S) in the presence or absence of 4-aminimidazole-5-carboxamide (AIC).**
The particular reaction which is most sensitive to inhibition by the active form of 6-MP is apparently the first step in purine biosynthesis. This is implied by the experiments of McCollister et al. (51) in which 6-MP ribonucleotide was as effective as natural purine nucleotides in inhibiting the synthesis of phosphoribosylamine from 5-phosphoribosyl-1-pyrophosphate and glutamine, with cell-free preparations of pigeon liver. Gots and Gollub (32) and LePage and Jones (46) had previously obtained evidence for inhibition of some early step in the de novo pathway by 6-MP and other purine analogs. Thus, the primary action of 6-MP which inhibits the growth of cells can be attributed to pseudo-feedback inhibition which mimics the role of natural purine nucleotides in the regulatory control of purine biosynthesis.

Modification of Folic Acid Metabolism

It is not my intention to review pharmacologic and biochemical studies on the folic acid analogs, since this subject was treated in detail by Bertino (5) and Werkheiser (76) and will be up-dated by their papers at this conference. However, it is consistent with the theme developed in this presentation to ask which of the many biosynthetic reactions requiring folic acid cofactors limits growth of leukemia cells when the supply of these cofactors is curtailed. The action of 4-amino analogs in creating an acute deficiency of the tetrahydrofolate cofactors by shutting off the reduction of folic acid has long been a matter of intensive study (5, 57, 76). Disclosure of the mechanism of action of amethopterin requires elucidation of the most sensitive or most critical enzyme with which the drug combines; nonetheless the impairment of cellular growth must be attributed to the resulting inadequacy of certain carbon-transfer reactions. It is my impression that the intensive focus of attention on the 4-amino analogs, although appropriate for the most potent and most useful type, has been at the expense of overlooking what might be accomplished by interfering with folic acid metabolism in other ways. It is for this reason that we have pursued for several years the study of the effects on experimental tumors of a diet-induced, as well as an amethopterin-induced, deficiency of folic acid.

An example of the selective impairment of tumor growth resulting from dietary deprivation of folic acid deserves mention because of its implications. Walker carcinoma 256 fails to grow in rats which are so mildly deficient in folic acid that their body weight continues to increase, yet this same tumor is refractory to nontoxic doses of amethopterin (64). This evidence that interference with folic acid metabolism impairs the growth of a tumor which is naturally refractory to amethopterin emphasizes the need for additional means of interfering with the functions of this vitamin and suggests that the spectrum of tumors susceptible to treatment may be extended to include some now classed as refractory.

In view of the many different carbon-transfer reactions requiring folate cofactors (26), it is reasonable to expect considerable variation in the degree to which the function of these individual enzymes is related to capacity for growth. Indeed, the accumulation of 5-amino-4-imidazolcarboxamide ribotide, but not glycinamide ribotide, in Escherichia coli or L1210 leukemic cells, when either is inhibited by amethopterin, indicates different degrees of impaired function (70, 71). Although 5-fluorodeoxyuridine is a remarkably potent inhibitor of thymidylate synthetase, it is not clear whether its limitations for the treatment of the leukemias are due to the pharmacodynamics of the drug or to the biochemical pattern of the leukemic leukocytes (39). The marked inhibition of formate-14C incorporation into DNA thymine in human chronic granulocytic leukemia leukocytes by amethopterin in vitro does not correlate with the ineffectiveness of this drug for this type of leukemia (75). In cultures of S-180 which are sensitive to amethopterin, hypoxanthine decreases the toxicity of the drug by 2-fold, whereas thymidine has no such effect (36). This evidence indicates that purines, rather than thymidine, are the limiting products of the 1-carbon transfer reactions reflected by the rate of growth of these cells. This is not surprising when the many functions of purines as cofactors and components of both RNA and DNA are compared with the restricted role of thymine as a constituent of DNA. Lieberman and Ove (48) have calculated that the formation of 1 rabbit liver cell in a culture requires some 15 times more adenine than thymidine. Thus, in addition to the impairment of growth of cells by amethopterin- or diet-induced deficiency of folic acid cofactors, comparable inhibition of growth may be achieved by analogs of tetrahydrofolate which can act on the most critical carbon-transfer reactions. The logic of this is as obvious as the need for such compounds.

Although inhibition of critical enzymes may determine the degree of growth impairment by amethopterin, this is now recognized as 1 piece in the mosaic peculiar to the response of each cell type. Factors controlling the pharmacodynamics of this drug and the significance of cellular uptake were discussed by Werkheiser (76) with reference to the selectivity of inhibition. A unique model system used by Hakala (33) allows detailed analysis of the rates of cellular entry and exit of amethopterin. The large amounts of folate reductase associated with the development of resistance to amethopterin in S-180 cells in culture (36) act as an intracellular trap for amethopterin, thus permitting kinetic measurements of cellular influx and efflux of the drug. In resistant cells the concentration of the drug in the medium is insufficient to inhibit growth, because the rate of influx of amethopterin does not keep up with the rate of formation of folate reductase. Thus, the rate of increase of this enzyme, which is determined by the enzyme content, the generation time of the cells, and the rate of induction, is such that some free enzyme remains functional. The data indicating that the rate constant for efflux of amethopterin greatly exceeds that for influx are of particular interest (33). Consequently, consideration should be given to agents capable of inhibiting cellular efflux of drugs as well as to those capable of accelerating their entry. Although the relevance of this model system to the transport of the drug in leukemic cells in vivo remains to be determined, nonetheless the need for such studies related to the means of modifying the intracellular concentration of the drug deserves emphasis.
DRUG ACTIVATION AND INACTIVATION

The distinction between drug potency and drug selectivity is well recognized. Various derivatives of cortisol are much more potent than the natural hormone, but there is no evidence that the spectrum of neoplasms susceptible to treatment has been extended. However, structural modifications which may distinguish between mineralocorticoid and glucocorticoid activities have been studied extensively (24, 25). One of the factors contributing to the increased potency of the synthetic glucocorticoids is their reduced susceptibility to enzymatic inactivation (19).

The wide range in potency of various corticosteroids when tested against certain cells in culture indicates a direct cytotoxic action without dependence upon metabolic alteration in the liver. In general, cytotoxicity is associated with anti-inflammatory and glucocorticoid activity (11, 27, 40). Also, thymolytic activity is correlated with glycogenic potency (60). A corticosteroid of increased potency based on its cytotoxicity for cultures of leukemia L5178Y was found to have higher host toxicity (40). Unlike the rather uniform sensitivity of different cell lines in culture to various purine, pyrimidine, or vitamin antagonists, the range in sensitivity to corticosteroids varies more than 100,000-fold among cells of different origin (65). These steroid-sensitive and -refractory cell lines should be very useful in attempting to explain the selective response of lymphoid neoplasms to this class of hormones.

Disclosure of the metabolic reactions which rapidly inactivate 6-MP in vivo led to the design of derivatives which protect the sulfhydryl group from oxidation and to the inhibition of xanthine oxidase as a means of slowing the formation of 6-thiouric acid. The 8-(1-methyl-4-nitro-5-imidazolyl) derivatives of 6-MP and 6-thioguanine have a higher chemotherapeutic index than the parent compound in experimental studies (14, 15). The xanthine oxidase inhibitor, 4-hydroxypprazolo-3, 4-d-pyrimidine, substantially increases the chemotherapeutic index of 6-MP and 6-chloropurine (17). Preliminary studies in man have demonstrated the same metabolic pattern as that seen in experimental animals (16). Although it is not possible to define the basis for the increased chemotherapeutic effect, this work illustrates the usefulness of understanding and modifying the pharmacodynamics of an anti-leukemic agent.

Since activation of 6-MP involves formation of the ribonucleotide, knowledge of the metabolic fate of such nucleotides may lead to means of prolonging the presence of the active form of the drug in cells by interfering with catabolic enzymes. Another approach toward the same objective is to use nucleotide derivatives which are better able to enter cells. Such compounds may serve as precursors of nucleotide analogs if acted upon by a phosphodiesterase. Various phosphate esters have been studied in this regard, and among these certain dinucleoside phosphates are of some current interest. Thomas and Montgomery (69) prepared a compound in which 2 nucleosides of 6-MP are linked through the 5'-positions via phosphate. This compound inhibited the growth of a 6-MP-resistant cell line in culture (52), indicating the likelihood that activation by intracellular cleavage to the nucleotide can occur.

Several phosphate derivatives of FUDR were synthesized by Remy et al. (59). In studies on the inhibition of the incorporation of formate into DNA thymine in suspensions of Ehrlich ascites carcinoma cells, Mukherjee and Heidelberger (54) found that various derivatives of FUDR varied considerably in their potency. Dinucleoside phosphates containing FUDR were less potent than FUDR itself in this test system. Hall et al. (37) have synthesized a number of dinucleoside phosphates containing 5-fluorouracil, FUDR, or bases which are minor components of nucleic acids, and the biologic activity of these compounds is being studied in our laboratory. The component mononucleotide and nucleoside of 3-methylidipridylyl-(3' → 5')-3-methyluridine were without effect on the growth of mammalian cells in culture, whereas this dinucleoside phosphate was an effective inhibitor of their growth (37). Thus, it appears that the intact dinucleoside phosphate can enter these cells.

It is a reasonable concept that cells resistant to fluorouracil or FUDR may be inhibited by dinucleoside phosphates containing these analogs as components. Several strains of Streptococcus faecalis 8043 were studied as a suitable test system in this regard. Dinucleoside phosphates containing either fluorouracil or FUDR were much less active than the parent compounds in a strain of S. faecalis which is sensitive to fluorouracil or FUDR. When tested with strains which are highly resistant to fluorouracil or FUDR (6), complete cross-resistance to dinucleoside phosphates of this type was observed (A. Bloch, M. T. Hakala, R. H. Hall, and C. A. Nichol, unpublished data).

Since the permeability of mammalian cells to such compounds may be different, further tests with other systems are needed before the potentialities of compounds of this type can be evaluated. In the course of this work, our attention has been directed to the need for better data concerning the extent to which nucleotides and phosphorylated sugars can be taken up by cells.

Although various examples of structural changes intended to confer lipid solubility on anti-cancer agents should be included in this discussion, it can only be mentioned that in these cases also there is dependence upon activation. Thus, it becomes important to find out whether the advantage of improved penetrability of cellular membranes or the blood-brain barrier is nullified by inadequate conversion to an active drug.

MANIPULATION OF METABOLISM CONTROLLING GROWTH

During the past several years, compounds from every conceivable source have been screened for anti-cancer activity. The possibility must be faced that, among the next 100,000 compounds to be tested for anti-leukemic activity, no agent will be more active or more useful than those presently in hand. This is not pessimism. It is realism. An alternate strategy is needed since the discovery of a curative anti-leukemic agent does seem to be a different kind of problem than uncovering an extra-hard-to-find antibiotic. Certainly, the intensive search for new drugs should be continued and can be well afforded.
when compared with the present economic priority of weapons (13).

The repetitious nature of this presentation is intentional because, in my opinion, there is considerable reluctance to tune in the message signaled by the evidence at hand. The most effective agents in the treatment of acute leukemia are (a) a corticosteroid which can amplify beneficially certain hormonal regulatory systems when given in pharmacologic amounts, (b) a purine analog which mimics the normal feedback control by nucleotides in a manner which can shut down cellular biosynthesis of purines, and (c) a folic acid antagonist which creates an acute deficiency of a vitamin that is essential for normal hematopoiesis. Each of these agents can kill leukemic cells selectively, if not completely. This evidence can be accepted as a basis for reasonable hope that curative treatment is attainable. Indeed, this goal would be much closer were it not for the predictable occurrence of drug resistance.

Acute leukemia, whether resulting from a viral agent, carcinogen, ionizing radiation, or other cause, once the disease is manifest, is attributed to a deranged or unregulated metabolism of leukocytes which continue to divide. The pessimism expressed some years ago that interference with the function of essential vitamins could not be applied usefully as an approach to cancer chemotherapy is also directed to the recently discovered biosynthetic regulatory systems. Magasanik (40) has made the realistic appraisal that, since rapidly proliferating normal tissues and cancer cells presumably use the same biosynthetic and regulatory mechanisms, any agents capable of acting as strong feedback inhibitors or repressors would likely damage not only cancer cells but also normal tissues. This is apparently true, yet the selective effects of the anti-leukemic drugs describe cellular differences in hormonal responsiveness, biosynthetic regulation, and folic acid metabolism. These are the kinds of differences between normal and leukemic leukocytes with which we must work.

If our objective is stated as the intentional manipulation of metabolism controlling the growth of leukemic cells, then a number of different approaches to the problem can be defined, in addition to the intensive search based on random screening. Certainly, it is by the use of drugs more active or more selective than those available at present by which we hope to achieve more prolonged remissions without toxic side-effects. From this viewpoint, however, our attention is directed to the metabolic consequences of the presence of each drug rather than primarily to the mechanism of drug-receptor interaction or the incorporation of analogs into cellular structures. Any active agents, with due recognition of their usefulness and limitations, serve as signposts pointing to metabolic sites where interference can limit the growth of neoplasms. Some of the metabolic effects of the glucocorticoids can be duplicated by dietary modification of the availability of amino acids. Leathem (45) has pointed out that little attention has been devoted to the possibility that dietary protein can modify adrenal steroid action. Goodlad and Munro (30) observed that the catabolic action of cortisone on the body as a whole was independent of the protein and energy content of diets fed to rats. However, the information needed concerns the selective catabolic action on lymphoid neoplastic cells. The extent to which the anti-leukemic effects of the corticosteroids may be amplified by manipulation of protein metabolism or the induction of amino acid imbalances has not been evaluated.

The pseudo-feedback inhibition of purine biosynthesis by 6-MP not only indicates the need to identify the structural requirements for this activity but also suggests that means of controlling the rates of interconversion within purine nucleotide pools or creating a shortage of glutamine or phosphoribosylpyrophosphate may amplify the potency and perhaps the selectivity of nucleotide analogs.

In addition to the type of interference with folic acid metabolism accomplished by the 4-amino analogs, the selective interference with carbon-transfer reactions requiring different folate cofactors (26) is receiving increasing attention. Baker et al. (1–3) have undertaken the synthesis of compounds designed as selective irreversible inhibitors of such reactions. The evidence presented recently by Goodman et al. (31), showing that homotetrahydrofolic acid selectively inhibits thymidylate synthetase, affords encouragement that this approach may be useful. Also, the type of compounds which are substrates for folate reductase, such as homofolic acid (31) or 10-methylfolic acid (82), may have special application when resistance to amethopterin is accompanied by the presence of extraordinary amounts of this enzyme.

In the case of compounds characterized as weak competitive inhibitors, control of the level of folate cofactors may determine their effectiveness. This may not only require dietary control of vitamin intake but might also be accomplished by analogs devoid of vitamin activity which can displace folic acid from cells as demonstrated by Johns and Pinderleith (42). Analogs capable of displacing tetrahydrofolate cofactors may accelerate inactivation or excretion of the vitamin, thus permitting a controlled degree of folic acid deficiency. With the intent to manipulate folic acid metabolism, the availability of precursors of the 1-carbon pool could be restricted, or the administration of compounds which would serve as acceptors of available 1-carbon units may serve as an adjunct to the administration of folic acid antagonists. Knowledge of nutritional requirements for man and improved palatability of diets of known composition, such as those widely used as a means of controlling body weight, now make clinical studies involving detailed regulation of diet more feasible (28).

**Combination Chemotherapy**

In addition to whatever may be accomplished by applying dietary control or metabolic stresses along with the use of corticosteroids, 6-MP, or amethopterin, more effective treatment will require the use of new drugs which can impinge on other metabolic pathways or other functional sites. Possibly the failure to achieve synergism by combining 6-MP and amethopterin in the treatment of acute leukemia (21, 22) may be due to the functional impairment by each drug of the same metabolic circuit involving the biosynthesis of purines. The effectiveness of treatment is enhanced by combinations of corticosteroids with...
other anti-leukemic agents (20, 23). Other speakers at
this conference will deal with the curative potential of
appropriate combinations of effective agents.

There is no doubt that random screening of synthetic or
natural compounds can disclose agents which may hit new
metabolic sites, thereby impairing the growth of leukemic
cells, but the yield has been small. With the intention of
interfering with metabolism in the manner represented by
the most active agents, several practical suggestions can
delineate.

1. Biochemical screening can be undertaken using those
enzymes for which there is evidence that changes in ac-
tivity are correlated with growth, development, or feed-
back inhibition. For example, there is evidence that
inhibition of deoxycytidylic deaminase by deoxyguanyl-
ate also inhibits the growth of chick embryos (66, 67).
Also, aspartate transcarbamylase is inhibited more ef-
effectively by purine than by pyrimidine, deoxyribonu-
cleosides, and deoxyribonucleotides (7, 8, 66). The selec-
tion and design of compounds based upon effectiveness as
inhibitors of such enzymes in suitable biochemical prep-
aratons could then be followed by tests for anti-tumor
activity and evaluation of their pharmacologic properties.

2. Pyridoxine deficiency is characterized, in experi-
mental animals (38) and in man (72), by lymphopenia,
which is accentuated by the administration of 4-deoxy-
pyridoxine. The effects of this weak competitive an-
tagonsit are readily prevented by the vitamin. Despite
the hazard that interference with pyridoxine metabolism
may adversely affect the functions of the central nervous
system as in the case of isonicotinic acid hydrazide (78),
anti-metabolites of pyridoxine capable of inducing lymph-
openia selectively may be useful in the treatment of
lymphocytic leukemias (63).

3. Combinations of drugs, one of which can affect
permeability or leakage of cell membranes, could be under-
taken with any suitable microbial or mammalian cell
system. Nystatin has a selective effect on leakage of K+
ions from yeast cells which causes inhibition of glycolysis
(68). Polylysine blocks release of proteins from Ehrlich
ascites cells but also causes leakage into the medium of
amino acids, K+, and adenosine monophosphate (44).
Information derived from such tests may be applied to
comparative studies of the susceptibility of different types
of cells. Many agents such as these may have limiting
toxicities upon parenteral administration. The nature of
their action which can be analyzed may be duplicated
by other drugs which may be more suitable for combination
with anti-leukemic agents.

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