Chemotherapy and Chemopraxis of Cancer, 1961*

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It is sobering to reflect that it was a scant 70 years ago when Ehrlich initiated our present chemotherapeutic era by a clinical trial, intravenous methylene blue for malaria, on two patients (38). By present mores, it was a modest venture, indeed; but he started a ball rolling which is still gaining speed and dimension. Many of Ehrlich’s ideas, technics, and even the lineal descendants of his compounds permeate cancer chemotherapy today.

In Table 1 are listed some cancer chemotherapeutic agents for systemic use and the respective diseases for which a therapeutic result can be expected in a proportion of patients with advanced cancer. Every drug on the list has arrived there in the brief ~0 years since Huggins and Hodges (50) introduced stilbestrol for the treatment of prostatic cancer and thereby sparked the modern era of chemotherapy. Indeed, every other compound on the list has been made only in the past ~0 years, with the exception of testosterone, which in its unesterified form has a long and noble history. They can be categorized as alkylating agents, hormones, antimetabolites, botanicals, antibiotics, and a synthetic compound of uncertain mode of action. The recent additions to this list are cyclophosphamide, which seems to induce less thrombocytopenia at equal levels of leukopenia than other alkylating agents on this list (82); 17-hydroxy-progesterone; 17-N-caproate, the progestational effect of which has extended the known spectrum of human neoplasms still partially responsive to normal mechanisms of growth regulation (51); desacetyl methylcolchicine, which can rapidly decrease the peripheral leukocyte count in chronic myelocytic leukemia (56); and vinblastine sulfate, which has induced responses in Hodgkin’s disease no longer responsive to alkylating agents (48, 102), and choriocarcinoma refractory to methotrexate (47); and o,p’-dichlorodiphenyl-chloroethylene, o,p’-DDD, active in suppressing adrenal cortical carcinoma (5).

The omission of urethan from the list reflects my indecision concerning the importance of its antineoplastic activity in man. For several of the compounds listed, congeners with minor structural changes exist for which no persuasive therapeutic superiority in cancer has been demonstrated and which do not enjoy as wide use as the compounds listed. Similarly, a large family of alkylating agents (19) and of androgenic steroids (80) are chemically active, but their inclusion on the list would not increase the breadth of effect nor improve the quality of therapy. For some compounds not listed, their recent introduction has denied them the corroborative test of time.

In addition to the active homologs of active compounds, there are an indefinite number of drugs which have been, and others which doubtless will be, tried, which do not elicit therapy in man. Such trials seem best to be described as chemopraxis. As a term, chemopraxis has the advantage of accuracy, and of avoiding the conceptual dilution of the sizeable community of chemotherapeutic compounds with ineffective drugs, for lack of another suitable classification. Drugs are eminently useful as pharmacological tools of clinical cancer investigation to distort the normal biological and biochemical relationships between the host and his tumor. The increasing emphasis that physician-investigators are placing on chemopraxis as a scientific calling, rather than chemotherapy as a clinical address, augurs well for the future of the treatment of cancer. Many drug studies in man are being conducted which, in addition to seeking better treatment, attempt to provide information of fundamental importance to the understanding of neoplastic disease in man. This review is aimed at assessing the fundamental information springing from clinical cancer chemotherapy today. I am indebted to a great many investigators for allowing me to use their own unpublished data, and data from group studies.

Methodical and systematic increases in our
knowledge, as they contribute to our understanding of the diseases we treat, provide scaffolding on which to mount the rational assaults of the future, and perhaps framework from which ultimate chemotherapy may stem. Ehrlich observed that difficult problems in chemotherapy should be considered in their long-term concepts—gutta cavat lapidem (drops wear away stone) (23).

Neoplasms in man, as in other animals, are heterogeneous populations of tumor cells, often with remarkably different responses to therapy in different parts of the population. It is commonplace to find a single tumor mass regressing during treatment with antitumor drugs while several other tumor masses do not regress; but ordinarily topurine, effective antileukemic agents, were given to groups of patients randomly allocated and divided by age and type of acute leukemia, into the three regimens shown in Chart 1. The study was divided into two phases, I and II, in which the opposite drug was given. The combination of the two drugs in full dose simultaneously was only given for one phase. Because each drug was given either early or late in the course of the disease, one could determine the effect of prior disease duration on response rate. Neither the prior disease duration nor prior therapeutic experience seemed to modify the remission rate in children with acute lymphocytic leukemia. In Phase I, 6-mercaptopurine total remission rate was 47 per cent and in

### Table 1

**Some Cancer Chemotherapeutic Compounds of Established Efficacy in a Proportion of Patients with the Neoplastic Diseases Listed Opposite**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Effect</th>
</tr>
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<tbody>
<tr>
<td>Mechlorethamine (nitrogen mustard, HN2)</td>
<td>Lymphomas</td>
</tr>
<tr>
<td>Cyclophosphamide (Cytoxan®)</td>
<td>Lymphomas</td>
</tr>
<tr>
<td>Chlorambucil</td>
<td>Chronic lymphocytic leukemia</td>
</tr>
<tr>
<td>ThiTEPA (triethylenethiophosphoramide)</td>
<td>Carcinoma of ovary, breast</td>
</tr>
<tr>
<td>Busulfan (Myleran®)</td>
<td>Chronic myelocytic leukemia</td>
</tr>
<tr>
<td>Stilbestrol</td>
<td>Carcinoma of prostate, breast</td>
</tr>
<tr>
<td>Testosterone propionate</td>
<td>Carcinoma of breast</td>
</tr>
<tr>
<td>17a-Hydroxyprogesterone caproate</td>
<td>Carcinoma of endometrium</td>
</tr>
<tr>
<td>Prednisone</td>
<td>Acute lymphocytic leukemia</td>
</tr>
<tr>
<td>Amethopterin (Methotrexate®)</td>
<td>Trophoblastic neoplasms</td>
</tr>
<tr>
<td>6-Mercaptopurine</td>
<td>Acute lymphocytic leukemia</td>
</tr>
<tr>
<td>Fluorouracil; fluorodeoxyuridine</td>
<td>Acute myelocytic leukemia</td>
</tr>
<tr>
<td>Desacetylmyeloleucine (Coleemid®)</td>
<td>Acute myelocytic leukemia</td>
</tr>
<tr>
<td>Vinblastine (vincleukoblastine, VLB)</td>
<td>Carcinoma of bowel</td>
</tr>
<tr>
<td>Actinomycin D</td>
<td>Chronic myelocytic leukemia</td>
</tr>
<tr>
<td>o,p'-DDD</td>
<td>Hodgkin's/HN2</td>
</tr>
<tr>
<td></td>
<td>Choriocarcinoma/Mtx</td>
</tr>
<tr>
<td></td>
<td>Embryonal rhabdomyosarcoma</td>
</tr>
<tr>
<td></td>
<td>Wilms tumor</td>
</tr>
<tr>
<td></td>
<td>Carcinoma of adrenal</td>
</tr>
</tbody>
</table>

This does not constitute the therapeutic effect for which we strive.

The emergence of resistance in experimental leukemia has been attributed to the presence of pre-existent resistant cells (54). Combination chemotherapy with two effective agents has been shown to provide therapeutic advantage in some mouse tumors (56), as it may in certain microbial diseases. Collateral sensitivity, increased sensitivity to a second drug in the presence of resistance to the first, was described in bacterial systems (90) and has been found also to be demonstrable for methotrexate in 6-mercaptopurine-resistant mouse leukemias (55). In an attempt to explore these biological and pharmacological parameters in acute leukemia of man, combination effects and collateral sensitivity, a study was undertaken by Leukemia Group B. Methotrexate and 6-mercaptopurine, effective antileukemic agents, were given to groups of patients randomly allocated and divided by age and type of acute leukemia, into the three regimens shown in Chart 1. The study was divided into two phases, I and II, in which the opposite drug was given. The combination of the two drugs in full dose simultaneously was only given for one phase. Because each drug was given either early or late in the course of the disease, one could determine the effect of prior disease duration on response rate. Neither the prior disease duration nor prior therapeutic experience seemed to modify the remission rate in children with acute lymphocytic leukemia. In Phase I, 6-mercaptopurine total remission rate was 47 per cent and in

### Chart 1

**Experimental design of a Leukemia study. Data of Leukemia Group B.**
Phase II, 44 per cent. In Phase I, the methotrexate response rate was 8 per cent and in Phase II, 22 per cent. Combination chemotherapy produced a 58 per cent total remission rate. The combination remission rate is the rate anticipated from additive effects of two active agents rather than from synergism. In those children with acute lymphocytic leukemia who sustained complete remission from the chemotherapy, suggestive evidence of the effective combination treatment was seen. Thus, the four longest remissions were present in the patients receiving 6-mercaptopurine plus methotrexate (Chart 2). Similarly, in adults, although significant differences were not generated with the small number of patients, long-lasting remissions were more frequent in patients receiving 6-mercaptopurine plus methotrexate than in those from either drug alone. The difference in remission experience was not translated into survival difference for the entire group of children with acute lymphocytic leukemia, and the survivals for the three treatment regimens were nearly identical. In adults, however, the suggestive but not statistically significant superiority of the combination of 6-mercaptopurine plus methotrexate over the two possible sequences in terms of survival is shown in Chart 3.

The extent of disease, as it influences therapeutic response, has been of interest to all chemotherapists, including Ehrlich. I am sure he would be interested in our latter-day propensity to attribute disease to cigarettes, and to try to cure it early, because 50 years ago he was far more concerned with disease caused by cigarette holders and attempts to cure it early. An aortic aneurysm in a recent patient of mine, however, was not so serious a threat to life as the bronchogenic carcinoma his wardmate had. Indeed, the man with the aneurysm outlived his predicted longevity by 8,600 per cent, after he was given 6 months to live and deferred from the draft, in World War I. He was treated with very effective chemotherapy, a new drug which Ehrlich and co-workers had reported.

The chemical treatment of advanced bronchogenic carcinoma, however, is unsatisfactory; and this led surgeons throughout this country to accept the preliminary observations on drugs administered at the time of surgery as a logical area in which to pursue their interests. It was demonstrated that “curable” patients might have circulating tumor cells and that the concentration of circulating tumor cells could be depressed by drugs (83, 89). Furthermore, surgeons working in the laboratory (81) had demonstrated the feasibility of enhancing chemotherapeutic response by surgical extirpation of the bulk of transplanted neoplastic tissue in rodents. A chance thus existed to treat micrometastases and circulating tumor cells before the mechanical and biochemical disorders and tissue destruction of advanced cancer were present. Well designed studies at this point a reasonable technicolor facsimile of a lady of easy virtue, smoking, should be called to mind.
were undertaken comparing nitrogen mustard and placebo injections at the time of surgery for carcinoma of the lung. It is of interest that no difference in survival was found, inasmuch as nitrogen mustard tends from time to time to evoke regression of an established tumor mass, particularly in oat-cell carcinoma. Approximately 1100 patients were studied in all. Entirely similar lack of effect on postoperative survival was seen for patients with carcinoma of the colon and rectum, whose study compound was thioTEPA. In gastric cancer treated with thioTEPA in the perisurgical period, no significant difference has appeared in the survival of control and treated patients.

In breast cancer, numerous investigators have reported some activity of thioTEPA against established metastatic disease. The data in Chart 4 are plotted by the life-table technic in terms of appearance of recurrent disease, as detected on 3-monthly follow-up visits. In Phase I of the study, because of considerable toxicity, which was occurring in other surgical adjuvant studies, although not prohibitive in breast cancer, drug doses were diminished throughout the adjuvant programs. At the lower dose of 0.6 mg/kg thioTEPA, a lesser difference exists between recurrence rate of treated and control patients (Chart 5). Here again, the major difference in recurrence is found between the control and treated groups in the premenopausal women, although in postmenopausal women with axillary nodes containing metastases a trend in favor of treatment also is occurring. The recurrences which have occurred so far in the first 404 postmenopausal women, in this second portion of the study, are 8 per cent in the control group and 6.9 per cent in the treatment. It is of note (Chart 6) that the Phase II control recurrence rate superimposes on the Phase I treatment rate. This could be explained by heterogeneity in the populations of breast cancer patients under study, chance, or some other

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Text continues with graphs and figures.
variable not yet apparent. Since a control group can behave this way by itself, however, and since the major differences in the study so far are seen in one segment of the population, the premenopausal group, and since it is still early in the follow-up, the utmost caution must be exercised, before changing our concepts of optimal treatment for primary breast cancer. More data, particularly on survival, are needed in this highly variable neoplasm.

Data are available on the first analysis of the series of 130 patients under study for a period up to 5 years by Cole and his associates. Patients with operable breast cancer were assigned at random to a control or treated group of 65 patients each. The treated group received melleschethamine, 0.4 mg/kg intravenously, in the immediate perisurgical period. The distribution of the two groups is similar with respect to menopausal status and axillary metastases. Deaths have occurred in 23 per cent of control patients versus 9.2 per cent of treated patients. The trend of treatment effect is recognizable in premenopausal and in postmenopausal women. In the surviving patients, recurrences are slightly higher in the control patients (35 per cent), both pre- and postmenopausally, than in those who received treatment (25 per cent). These data suggest a treatment effect, but until all these investigations are further along, it would not be prudent to consider them as other than an area of research.

Disillusionment is not in order, however. These studies have demonstrated that leukopenia of moderate degree in the low dose studies does not enhance the recurrence rate in breast cancer, nor does it compromise survival experience in carcinoma of the lung, or carcinoma of the bowel. An enviably efficient organization with rapid patient accrual has been established under the aegis of the Cancer Chemotherapy National Service Center. With the security of past experience in hand, I believe drugs with suggestively demonstrated activity against the homologous neoplasm in patients with advanced disease should appropriately obtain early controlled clinical trial in this type system. Although it is to be hoped that sufficiently sensitive biochemical tests will be discovered, allowing the physician to discriminate those patients who have micrometastases from those who are surgically cured, such a hope is unfulfilled as yet, and the wider application will be required.

Fluorouracil generated enormous interest in the clinic because of the beauty of the rationale, the animal tumor data, and the remarkable chemotherapeutic responses described in early reports (9, 20). Subsequent reports described lesser activity (11, 34). Availability of fluorodeoxyuridine and variable assessments of its clinical contribution (9, 62, 103) led Dr. Howard Skipper at a meeting on fluoropyrimidines to question whether the degree of toxicity to which different chemotherapists ordinarily took their patients might explain the differences in results observed, and, more fundamentally, whether marrow and gastrointestinal toxicity per se, as indicative of profound chemical alteration in cellular function, might be a nonspecific determinant of chemotherapeutic response. This problem suited the interests of the Eastern Solid Tumor Group, where a drug, fluorouracil, its presumed active metabolite, fluorodeoxyuridine, and another antimetabolite widely believed also to exert its major effect on inhibition of thymidyl acid biosynthesis, methotrexate, could be used. Appropriate doses were picked in an attempt to produce equal toxicity. Those neoplasms reported to have greatest susceptibility to the fluoropyrimidines were chosen for study. Then everything was coded. Drug solutions were the same volume and color, administered by the same schedule, and retreatments applied by an explicit protocol. Partial objective regressions were determined by collective assessment of the investigators, without code break. Patients were assigned to treatments I, II, or III by random allocation. The preliminary data on the first group of patients who have finished the study show some activity of all three drugs, although it appears least for compound III (Table 3). Thus, by blind subtraction, a mathematical technique appropriate for blinded data, at least one fluoropyrimidine compound is showing chemotherapeutic activity in this study. Our primary objective has not yet been fulfilled, however. The toxicity scores, where 1 equals mild and 4 equals fatal intoxication, are indeed ranked according to the therapeutic result, and the greatest effect was purchased at the greatest cost in toxicity. These data are preliminary.

The Cooperative Breast Cancer Group of the C.C.N.S.C. has studied advanced breast cancer in an effort to elicit maximum information of fundamental nature in addition to assaying active drugs

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(80). These investigators accepted testosterone propionate as a primary standard of activity. The data from their studies are the best available concerning hormone action in breast cancer. All their studies were performed on women with advancing breast cancer. Premenopausal women, and those within 1 year's cessation of menses, were surgically ovariectomized. Advancing cancer despite castration was prerequisite to inclusion into the study. The data demonstrated difference in responsiveness to testosterone propionate in those individuals who were ovariectomized less than 1 year previously, contrasted to those 1–5 years, or 5 years and more postmenopausally (Table 3). All these differences are highly significant. No significant increase in responsiveness to testosterone was seen at any time beyond the 5th year. Two androgens of the nearly 50 whose study has been undertaken have been found, after extended study, to possess activity approximately equal to that of testosterone propionate. Fluoxymesterone was confirmed as an oral androgen of choice.

Table 2

<table>
<thead>
<tr>
<th>Drug</th>
<th>I</th>
<th>II</th>
<th>III</th>
</tr>
</thead>
<tbody>
<tr>
<td>Disease</td>
<td>Response total</td>
<td>No. toxic</td>
<td>Toxic score</td>
</tr>
<tr>
<td>Ca. colon</td>
<td>4/12</td>
<td>9</td>
<td>1.3</td>
</tr>
<tr>
<td>Ca. breast</td>
<td>1/5</td>
<td>4</td>
<td>1.2</td>
</tr>
<tr>
<td>Miscell. ca.</td>
<td>1/6</td>
<td>4</td>
<td>1.5</td>
</tr>
<tr>
<td>Total</td>
<td>6/23</td>
<td>17</td>
<td>1.3</td>
</tr>
</tbody>
</table>

Table 3

<table>
<thead>
<tr>
<th>Hormonal Treatment of Advancing Metastatic Breast Cancer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cooperative Breast Cancer Group Data, March, 1961</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Response, per cent</th>
<th>Overall response (per cent)</th>
<th>Total cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Testosterone propionate</td>
<td>Ovariectomized, 1 yr.</td>
<td>1–5 years postmenopause</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>20</td>
</tr>
<tr>
<td>Fluoxymesterone</td>
<td>0</td>
<td>9</td>
</tr>
<tr>
<td>2-a-Methyl-dihydrotestosterone</td>
<td>6</td>
<td>22</td>
</tr>
<tr>
<td>Total</td>
<td>7</td>
<td>19</td>
</tr>
</tbody>
</table>

*Response excluding initial report. See text.

2-a-Methyl-dihydrotestosterone is an intriguing compound. Four studies of its activity have been made and are combined in Table 3; the first was considerably more favorable than the subsequent three (10), although the 19 per cent regression rate of the three confirmatory studies is of the order of magnitude of testosterone propionate. It is of note that the compound in the dose administered was not thought to be nearly so masculinizing as testosterone propionate, and the urinary excretion of gonadotrophic hormone was not altered (9). Both masculinization and decreased gonadotrophin had been part of the prior clinical technic for choosing effective androgens for the treatment of breast cancer. To emasculate these partial misconceptions is to broaden the potential for finding new steroidal activities which may not be potent androgens.

Other tests in progress by the several investigators of the Group are designed to study some of the potential metabolites of testosterone in an effort to identify a possibly more active compound. In the course of investigations with highly sub-

* P. Sheeh, personal communication, 1961.
stituted synthetic steroids, the group has found activities in small series of patients essentially equal to testosterone propionate for two oral androgens, two intramuscular androgens, and two progestational compounds (80). The implications these data have to concepts of tumor autonomy and hormonal interdependence are not without consequence. A similar examination of estrogenic and progestational structure and function appears worthy.

With possibly a single exception, trophoblastic neoplasms in a proportion of the women affected, all other chemotherapeutic successes in human neoplasms have eventually been terminated by recrudescence disease despite continuing drug administration. Increasing the systemic dose of drug is at best a transient stopgap, since the selective toxicity for the neoplasm is lost and greater host toxicity ensues. Resistance is not peculiar to our problems. Ehrlich used a triply-resistant trypanosomal strain “as a therapeutic sieve, to separate what is the same from what is different” (23).

The mechanism of action of chemotherapeutic compounds in man, or in model systems thought to be representative of man (and some of these systems stretch far back on the biological scale) are essential to an understanding of resistance, and have continued to fix the attention of many workers.

The biologically relevant locus of action of the alkylating agents is still unproved (1, 16, 28, 33, 79, 91). The extremely high mutagenicity of ethylmethane sulfonate, CB-1528, a monofunctional derivative, has caused a re-inspection of the essentiality of bifunctional groupings for mutagenesis, and, by analogy, for tumor inhibition (100). The cross-linking hypothesis (35) has therefore been called into question. Furthermore, Alexander and Lett (1) have pointed out that ethylmethane sulfonate does not 7-alkylate guanine, making this reaction an unlikely general mechanism. Reported interference by alkylating agents with DPN synthesis (53) has recently been observed to follow gross evidence of cellular damage and therefore seems to be a secondary effect (37). A newly described activity of busulfan and of aniline mustard to combine with and extract a sulfhydryl group from protein, thus leaving behind a protein with a changed amino acid sequence (Chart 7), is of

**DETHIOLATION REACTION**

**ROBERTS AND WARWICK**

CHART 7.—Busulfan metabolism showing possibility for alteration in structure of protein molecule by dethiolation (74)
interest but has yet to be demonstrated as the mechanism of tumor suppression (74, 75).

A recent study of cyclophosphamide confirmed its nontoxic properties until metabolic alteration occurred. In rodents, the liver is at least one site where this metabolism can transpire (31). The novel mechanism of activation of cyclophosphamide and its superlative potency, and the wide spectrum of action in transplanted and autochthonous rodent tumors (65), led to a comparative estimate of its potency in a controlled trial against nitrogen mustard, the clinical standard, in a wide spectrum of human neoplastic diseases. The preliminary data of the Eastern Solid Tumor Group in Table 4 show that these two alkylating agents, Compound I and Compound II, are running cheek by jowl in every category. In equally sick patients, at equivalent levels of severity of gastrointestinal and hematological toxicity, the objective tumor regressions, and out of this number, the patients who also sustained temporary clinical well being, are just about the same. These are preliminary data. Information on uracil mustard will also be available in the final data. The biochemical or pharmacological explanation for this marked disparity of rodent and human assay would be of substantial aid in designing new drugs and in picking the proper ones for clinical trial.

The mechanisms of action of corticosteroids, which produce temporary effects of extraordinary magnitude in acute lymphocytic leukemia, have seen little elucidation in the 15 years of their use. Rosen and co-workers have described marked increase in alanine-a-ketoglutarate transaminase in Walker carcinoma and thymic tissue in rats treated with cortisol (76). More recently, data were presented that an established cortisol-sensitive lymphocytic neoplasm, P-1798, shows a 10- to 15-fold increase in alanine transaminase activity, whereas a cortisol-resistant line of the same tumor failed to show any change of enzyme activity on cortisol treatment (78). Here, too, is a biochemical tool which may make chemotherapeutic effects more understandable.

Recognition that purine analogs including 6-MP were anabolized to ribonucleotides by reaction with 5-phosphoribosylpyrophosphate (58, 94) led to ready appreciation that this might be the active form of the drug, the product of a lethal synthesis (70) (Chart 8). One major postulated mechanism of resistance to purine analogs, loss of the appropriate enzyme for conversion of the analog from purine base to ribonucleotide, was therefore sought (13). Conclusive data have been presented not only for bacteria, but for mammalian cells, demonstrating that the 6-mercaptopurine-resistant cell can have a markedly decreased capacity to catalyze the formation of 6-mercaptopurine ribotide, because of a decrease in inosinic acid pyrophosphorylase activity (15). Preliminary data in man demonstrate that the same pathway exists in leukemic cells, and changes in its activity in resistance are being sought (14).

Amethopterin (Methotrexate) has induced complete remissions in 30 of 63 women with choriocarcinoma and other metastatic trophoblastic neoplasms. Twenty-six of these complete remissions have lasted more than a year, some up to 5 years. These data of Hertz constitute one of the major accomplishments of cancer chemotherapy (46). Ancillary immunologic considerations in the mechanism of this effect have yet to be defined, but are possibly important (18). The activity of the antifolics in acute leukemia of childhood is of lesser degree. Clinical refractoriness to methotrexate eventually develops in all acute leukemias, and in some women with choriocarcinoma.

In the course of resistance to folic acid antagonists developing in mammalian cell cultures, a markedly increased content of the enzyme folic reductase has been found in the cells (30, 39).
This protein binds with folic acid antagonists almost irreversibly, and effectively sequesters the drug within the cell. Although the astronomical levels of resistance found in some cultured cells (29) could not be attributable solely to increased content of folic reductase, the minor increase of resistance necessary to vitiate the greater susceptibility of the leukemic cell compared with the host cell in vivo is entirely within possibility. Recent investigations of Bertino et al. (6) have shown dihydrofolic reductase in the leukocytes of all of twelve cases of acute leukemia, but not in normal leukocytes or in those of patients with chronic lymphocytic leukemia. The human enzyme was inhibited at the same extremely low drug level as in other systems studied, leading to the conclusion that it was a primary target for folic antagonist therapy. Of six patients with acute leukemia treated with amethopterin, enzyme activity increased as much as five- to twenty-fold in association with the development of resistance (7, 8). This increased enzyme might tightly bind whatever drug gained entry to the cell, and the susceptibility of the patients' normal cells to folic antagonist intoxication precludes a higher dose. These exciting findings are an admirable display of early translation of new chemotherapeutic research technics to the bedside.

Major efforts of several productive investigators have been devoted to the area of pyrimidine attributable to deficiency of uridine kinase with consequent inability to make azauridylic acid, the product of the lethal synthesis (67). The further normal pyrimidine metabolic pathway is schematically illustrated in Chart 10. The meticulous studies of Heidelberger and his associates have pinpointed one of the principal tumor inhibitory effects of the fluoropyrimidines, by way of the synthetic route shown, to inhibition of thymidylate synthetase (45). The focalization of drug effect on thymidylate synthetase in man can apparently be made much sharper by slow continuous low dose infusions of FUdR, probably thus avoiding spillover into other metabolic pathways. Prevention of the toxic effects of FUdR by infusion of thymidylate, the product of the in-
hibited reaction, has been demonstrated under these conditions (61, 89).

The other 5-halogenated pyrimidine nucleosides (I, Br, Cl) can substitute for thymidine and compete with it in the phosphorylation reactions and in incorporation into DNA (21). They are rather unstable in vivo, but IUdR nonetheless has some chemotherapeutic activity in man (98). No important therapeutic advantage derived from using FUdR and IUdR, potentially sequential blockers, in combination in man (66, 89). When IUdR, BUdR, and CUdR are incorporated into DNA, the susceptibility of cultured cells to radiation is markedly increased (25). Preliminary clinical trials to study this combined treatment have started (98). 11

Regionalization of drug administration by arterial infusion was attempted many years ago (52, 84). Recent investigations in this direction have included attempted biochemical reversal, usually incomplete, of the drug activity in the general circulation (86, 88). The technic may accomplish, by attaining selective toxicity through mechanically induced differential drug concentration, what cannot yet be accomplished by systemic drug administration. Sullivan and co-workers have described objective regressions in over half their patients who received intra-arterial methotrexate for epidermoid carcinoma of the head and neck or carcinoma of the cervix (87, 88). In eight patients (of a series of more than 50) complete regression of tumor has been seen, transiently or for sustained periods (85). Regional protection of marrow by chemical inactivation of alkylating agents (41), or by supply of a potential reservoir of leukemic cells, by eliminating a source for repopulating the marrow, may improve the over-all therapy of leukemia.

Other approaches to chemical control of tumor growth, not classically chemotherapy, merit mention. Dietary deficiency of folic acid in rats may cause remarkable inhibition of tumor growth. 13 Other workers (77) have shown marked suppression of Walker 256 carcinosarcoma growth by folic acid dietary deficiency which could not be duplicated by administration of folic acid antagonists. Dietary deficiency of pyridoxine is far more effective in Sarcoma 180, in causing complete regression of tumors, than any of the known pyridoxine antagonists (59). Tumor inhibition from dietary deficiency of pyridoxine also occurs in several other rodent tumors (60). Clinical explorations in these directions have been few and


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PYRIMIDINE NUCLEOTIDE METABOLISM

CHART 10.—Pyrimidine nucleotide metabolic pathways and the principal loci of action of pyrimidine antimitabolites on synthesis of thymidylate and on its subsequent phosphorylation. Abbreviations: C = cytosine; U = uracil; FU = fluorouracil; T = thymine; IU = iodouracil; dR = deoxyribose; P = phosphate; DNA = deoxyribonucleic acid.
incomplete (92, 99, 95) and provide a possible area for therapeutic gain.

Attempts to alter neoplastic cellular behavior toward normal are worthy of intensified interest and further trial. Attempts at enzymatic reflection (4) and the continuing search for growth-regulating substances in biological materials are ready examples. In many, if not all, neoplasms, residual characteristics of normal cellular behavior are prominent. That the growth of cancer is, by definition, not normally controlled, is not to say that it is completely uncontrolled.

The progressively greater attention being devoted to creating biological congruence between specific human cancers and the preclinical systems used to select drugs for exploratory clinical trials are worthy. Tissues with several biochemical characteristics in common should have pharmacologic and chemotherapeutic characteristics in common more often than two widely unrelated tissues.

"If there is no gain the loss is obvious"

—an old Italian proverb—

Merrill Lynch, Pierce, Fenner & Smith Inc.

Thus, the recognizably close biochemical and pathological approximation of a spectrum of murine myelomas to a spectrum of human myelomas (26, 71) commends it well for use as a selecting device for drugs for human myeloma, which has started (42). Similarly, neoplasms of homologous organs (88) and melanomas (73) are coming progressively more into their own with a hope for specific screening. Continuing awareness of immunologic considerations, and the possible laboratory artifacts of the transplanted tumor, have prompted some to undertake the study of chemical control of autochthonous tumors (40). Inhibition and partial regression of carcinogen-induced sarcomas of the subcutaneous tissues of rats and mice demonstrate that these technics are feasible. Autochthonous neoplasms of other tissues and organs and species will be welcome additions and are deserving of intensive work. Technics to study a patient's own excised neoplasm and to use it for selection of an appropriate drug need tremendous developmental research effort. I believe that the efforts to improve the profit margin of our product, cancer research, have been commented on in another context by Merrill and co-workers, from their laboratory on Wall Street (Chart 11).

Finally, it is impressive how many of the chemotherapeutically useful drugs were tried in neoplastic disease because of pronounced effects recognized first on a homologous normal tissue: Sulfur mustard and nitrogen mustard on marrow function and lymph node contents of man and experimental animal (33), adrenal cortical compounds and corticotropin on thymic and lymph node involution of the rat (29, 99), and o,p'-DDD on the normal dog adrenal gland (64), to name but a few.

The welcome greater emphasis recently on primates in cancer research (particularly the compact models) can hopefully provide a desirable species for intensive effort in seeking chemicals which produce specific tissue damage. Such compounds might be even more toxic to a neoplasm arising from that tissue.

Now, in seeking an appropriate quotation to use in conclusion, I could not cut my selection below three, so I will use all, for the benefit of those who have already hoped twice that I'd stop.

First, Dr. Heidelberger wrote cogent advice that is fitting thought for every day in the lab and in the clinic: "... These experiments show that the mechanism worked out in vitro obtains in the living animals at chemotherapeutic doses. The importance of this cannot be over-emphasized, because the literature is replete with reports of biochemical effects of drugs, which are inferred to be relevant to mechanism of action without any information as to the relationship of the biochemical effect to the chemotherapeutic result obtained in the intact animal. It is incumbent upon those working in this field to demonstrate unequivocally that the effects of drugs on biochemical systems, from which mechanistic significance is drawn, obtain in the whole animal at normal chemotherapeutic doses" (44).

Second, I shall paraphrase Dr. Bradford Patter-son with a thought for every time a page is turned in a new abstract journal: "We should be concerned not only with magic bullets, but in learning how to aim the gun" (69).

And, finally, of course, is Ehrlich, whose knowledge of how to finish a speech was flawless: "Indeed, from the very origin of the act of healing, chemotherapy has been in existence, since almost all the ingredients we employ are chemicals: on the other hand, experimental chemotherapy could..."
only develop in a fruitful manner in modern times as a result of all the pioneer work. But, here also, it has been proved that the four most important factors are patience, skill, luck, and last, but not least, money."

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