Summary of Informal Discussion on the Basis for Future Approaches to Cancer Chemotherapy

H. George Mandel

Department of Pharmacology, The George Washington University, School of Medicine, Washington, D. C. 20005

Following the presentations on the design of new therapeutic agents, Dr. Mandel opened the discussion by remarking on the extensive progress that has been made in the past years in directing the synthesis of new drugs. Whereas formerly the availability of new chemicals frequently was limited by the technics for preparation of compounds of new structures or modified functional groups, our improved understanding of the requisites for interaction with specific enzymes now is playing an ever increasing role in the design of chemical structures with greater therapeutic usefulness.

Dr. Schepartz agreed with Dr. Heidelberger that greater effort should be devoted to the synthesis of antitumor agents more specifically than to alkylating agents. In both fields, however, new and useful drugs have emerged when the basic structures of the compounds were altered substantially, whereas empirical and minor changes, such as those in side chains, have been relatively unfruitful. Even with the alkylating agents, which have been quite thoroughly explored, new structures with altered biologic activity might still be uncovered. For instance, the large number of methanesulfonates in the past have demonstrated rather restricted activity in experimental tumor systems or in man, but more recent derivatives such as those from Dr. Sakurai’s laboratory, have begun to show high activity against L1210 leukemia cells.

Dr. Sakurai provided additional information on these drugs which are derivatives of aminoglycols:

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\begin{align*}
\text{CH}_3\text{N} & \to \text{CH}_3\text{CH}_2\text{CH}_2\text{O}_2\text{SCH}_3 & \text{I (No. 838, NSC 84641)} \\
\text{H}\text{N} & \to \text{CH}_3\text{CH}_2\text{CH}_2\text{O}_2\text{SCH}_3 & \text{II (No. 864, NSC 102627)}
\end{align*}
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The compounds differ only by a methyl group on the nitrogen. The two drugs were equally effective against L1210, rat ascites hepatoma, AH13, and AH272 cells. Compound I was effective against Yoshida sarcoma and AH130, but almost ineffective against AH66, AH7974, and C1498 tumors. By contrast, Compound II strongly inhibited AH7974, AH66, and C1498 neoplasms. It is remarkable that, of more than 250 alkylating agents prepared in his laboratory, Compound II was the first one to affect the AH7974 and AH66 tumors, which are normally extremely refractory to drugs. Compound II was not very inhibitory to the Yoshida sarcoma or to AH130 cells, which usually respond to alkylating agents.

Although the acute toxicities of Compounds I and II to mice and rats were similar, chronic toxicity of Compound I was about twice that of II. The toxicity of I to bone marrow and spleen was greater than that of II. Almost three times the levels of radioactivity were recovered in AH66 cells following the administration of labeled II compared to I.

Because of the altered antitumor spectrum resulting from the slight modification in chemical structure, Dr. Sakurai also felt that further exploration among alkylating agents might still be profitable. Preliminary clinical evaluation of I and II in leukemic patients appeared promising.

Dr. Bertino referred to Dr. Baker’s compounds as representing a marriage between antitumor agents and alkylating agents. The compounds, as antitumor agents, occupy an enzyme site and then irreversibly alkylate outside the active center of the molecule. These compounds, therefore, may detect amino acid differences in enzymes away from the active site and, depending on the composition of the enzyme, may show species and perhaps even organ specificity. It is now of major importance to determine whether these drugs selectively exploit differences between normal and malignant tissue in man.

Dr. Young commented on the investigations of Dr. Connors regarding tumor levels of \( \beta \)-glucuronidase which reactivates alkylating agents selectively. He has been studying \( \beta \)-glucuronidase content of tumors at a histochemical level, concentrating primarily on tumors of the reticuloendothelial system and solid tumors. While tumor cells of most patients with Hodgkin’s disease had only slight \( \beta \)-glucuronidase activity, neoplasms of the few patients studied with myeloma and breast cancer showed significant enzyme activity. Dr. Young recommended that clinical studies should be coupled with biochemical and histochemical assays.

The discussion then dealt with newer approaches in cancer chemotherapy. Dr. Burchenal commented on the differences among the available commercial preparations of \( E. \) coli asparaginase. After a single intravenous injection to patients, asparaginase from Squibb and Merck companies exhibited plasma half-lives of 20 hours, whereas that produced by Bayer was only 10–12 hours. Recently, however, the latter company has in some unknown manner chemically modified its product to reduce antigenicity. The new enzyme preparation also has a half-life of 20 hours and unaltered therapeutic properties. All enzyme samples were found to have identical antileukemic activity in animals. No information is available on the relative antigenicity of the Bayer preparations, but the possibilities of further modifications imply that, as in the case of the semisynthetic penicillins, new preparations with additional therapeutic benefits are possible.
Dr. Potter commented on the great diversity among tumor tissues all derived from the same type of normal cells, i.e., parenchymal liver cells. Variations such as those in tumor levels of catabolizing enzymes for amino acids, as had been discussed by Dr. Handschumacher, are also exhibited with respect to transport of two amino acid analogs, cycloleucine and aminobutyric acid. These compounds, which lack an alpha hydrogen, are not metabolized or incorporated into protein and may be excreted at the rate of only one percent per day. The compounds are actively transported from blood into tissues, and ouabain inhibits the transport system. The transport system is quite specific and is unaffected by uridine or thymidine; only valine antagonized cycloleucine transport. Studies in Dr. Potter’s laboratory have shown transport of the analogs into and out of the liver to be on a rhythmic 24-hour cycle which is on a reciprocal basis with that in intestinal mucosa and perhaps other tissue.

The steady state levels of the amino acid analogs, measured as the ratio of concentrations in hepatoma to that in blood, showed a wide range, indicating great variations in the transport ability of tumor tissues. Because of the slow excretion of the drugs, these measurements can readily be made following injection of the analogs one or two days prior to the experiment. He suggested that this transport system might be correlated with amino acid catabolism in particular tumor lines, and thus it might serve to predict sensitivity to asparaginase and other drugs. The transport procedure might also be useful for studying permeability changes of tumors since alteration in the steady state concentrations of the amino acid analogs in the tumor cells can be readily observed under various conditions or treatments.

Dr. Weiss raised a question regarding increased therapeutic effectiveness resulting from improved penetration of carcinostatic drugs. Many attempts have been made to induce a Schwartzman type of reaction at the site of a tumor focus, in the hope of selectively destroying the focus of neoplastic cells. These attempts have never been therapeutically useful because of toxicity. Would it be possible to increase the permeability of the vascular bed of a tumor by a Schwartzman-like reaction quantitatively insufficient to completely destroy the tumor cells but adequate to facilitate ingress and to permit concentration of a cytotoxic agent in the tumor mass itself?

Although there was no specific answer to the question, Dr. Leighton suggested that one aspect of hyperthermic chemotherapy of metastatic melanoma probably involved increased permeability of the blood vessels in the foci of the malignant nodules and improved drug delivery to the melanoma masses.

Dr. Garattini noted that there already is considerable information available on altering permeability by the use of drugs such as histamine, serotonin, bradykinin, and other polypeptides. It may be possible to affect selectively capillaries of different diameter, and so permeability might be altered in specific parts of the vascular bed. Such an approach may be useful when chemotherapeutic agents are bound to proteins and, therefore, are not readily available to the cancer cells.

Considerable progress has been made at the biochemical level in studying connective tissue which plays an important role in permitting the spread of tumors. Biosynthesis and degradation of collagen are now better understood, and key enzymes such as proline hydroxylase and collagenase may be activated or inhibited by several factors. The activity of proline hydroxylase, the enzyme which hydroxylates proline in polypeptides to 4-hydroxyproline, is increased several fold in skin during certain types of chronic inflammation and during the growth of transplantable tumors.

Dr. Leighton emphasized that the enzymatic systems for collagen synthesis and degradation in tumors are probably the same ones found in normal growth and development, as well as in inflammation and wound repair. However, he suggested that the programming for these enzymatic systems in the continuing proliferation of the interstitial compartment of carcinomas appears to differ from the normal sequence of connective tissue changes seen in wound repair and scar formation.

Furthermore, there is great diversity in the structure and spatial distribution of the interstitial compartment of different tumors, even though the pattern is often characteristic for a particular tumor. No information is available at this time as to where the integrative regulation, i.e., the programming, for these characteristic patterns resides and how these controls are mediated. The identification and pharmacologic modification of such regulatory systems may be expected to influence the growth of carcinomas and under some conditions reduce the rate of tumor cell replication.

Dr. Karrer reported on some of his observations regarding vascularization. In the Lewis lung carcinoma implanted in the leg of BDF1 mice, chemotherapy 12 days after implantation had little influence on the growth of the primary tumor. Following amputation of the tumor-bearing legs, it was possible to inhibit growth of metastases in the lungs with chemotherapy, but regrowth of tumor in the area of amputation could not be prevented. Thus, in the same animal, the effect of chemotherapy varied, depending on whether it was directed at a primary solid tumor, metastases in the lungs, or tumor cells in the area of amputation. The small lung metastases have been demonstrated histologically to be in close contact with capillaries; the residual tumor cells in the area of surgery exist under different conditions of vascularization, and other local reactions, such as wound healing and inflammation, may also interfere with tumor growth. Altogether, the effectiveness of chemotherapy is a function not only of the number of tumor cells, but also their localization, the nature of the tumor, the particular environment of the tumor cells, and the concentration of drug reaching the tumor cells.

In relation to Dr. Leighton’s remarks on directing chemotherapy not only against tumor cells, but also the surrounding interstitial tissues and the vascular system, Dr. Mihich commented on some recent attempts in his laboratory of isolating temporarily the blood flow of a tumor from that of the host by administering 5-hydroxytryptamine (M. De los Angeles Contreras, W. F. Bale, I. L. Spar, and R. W. Helmkamp, Federation Proc., 27: 608, 1968). Antimetabolites are currently being administered, and thereafter 5-hydroxytryptamine and the corresponding normal metabolite are provided to selectively protect the host tissues while the circulation of the rescue agent to the tumor is inhibited. Results are still incomplete, however.
Regarding exploitations of differences between tumor and normal cells, especially at the macromolecular level, Dr. Busch mentioned two possibilities for RNA defects in cancer cells. Cancer cells may have specialized messenger readouts which differ from those of other cells or, alternatively, they may have normal readouts which vary quantitatively from those of other cells, so that accumulated intermediates keep the genome depressed. However, great technical problems still exist in the fractionation of messenger RNA, which encompasses a range in molecular weights from 200,000 up to many millions. Until now it has not been possible to resolve the 35 S and 45 S RNA complexes, and specific hybridization technics have been unsuccessful in isolating or separating normal and tumor messenger RNA’s.

Dr. Busch also commented on his comparisons of RNA readouts in a variety of hepatomas and those of regenerating liver. In newly synthesized nuclear RNA, he uncovered differences in composition ranging from the very GC-rich type of RNA, characteristic of the rapidly growing Morris hepatomas and Novikoff hepatomas, to the AU-rich RNA’s, characteristic of normal liver. The only constant changes found throughout the series of hepatomas were a low A and a high C content of the nucleolar RNA. In regenerating liver, throughout the early stages ranging from 3 to 20 hours of regeneration, there was no significant difference from that of normal liver in the nucleolar readouts or the whole nuclear readouts.

Dr. Heidelberger commented on selectivity of synchronizing tumor cells without a corresponding effect on normal cells and referred to the work of R. J. Martin and P. R. Schloerb (Cancer Res., 24: 1997–2000, 1964). By cyclically raising the body temperature of rats bearing Walker 256 tumor, these investigators have been able to synchronize tumor cells quite effectively over six-hour periods. Mitotic counts in the tumor sometimes reached peaks of five to ten times the normal values, whereas there was no variation in the intestine of the same rats. These results are particularly interesting because of the common belief that with circadian variations there is cycling in normal tissue, whereas, at least in some tumors, cycling does not take place. Dr. Heidelberger is examining this apparent selectively induced cycling of mitosis in a solid tumor because of its potential significance in chemotherapy.

Dr. Philips warned that the intestinal epithelium probably is an inappropriate system for studying mitosis in an animal being subjected to a stressful procedure because the intestinal mucosa is relatively resistant to adrenal corticoids. He recommended using tissues which respond to corticoids, such as lymph nodes or even regenerating liver.

Dr. Mihich recommended that greater efforts be made to attempt to modify the structure and/or the function of cell membranes. Modifications in the cell membrane may affect not only the transport of nutrients and drugs, but also cell contact phenomena, nuclear events leading to mitosis, and the degree of immunogenicity of cells. A modification of cell contact phenomena may affect the metastatic process. The effect of phytohemagglutinins on the metabolism of lymphocytes represents an example of the possible influence of phenomena occurring at the cell surface upon nuclear metabolism. The immunogenicity of cells may be modified not only chemically, by hapten-like substitutions, but also metabolically. For instance, E. A. Boyse, L. J. Old, E. Stockert, and N. Shigeno, (Cancer Res., 28: 1280–1287, 1968) showed that the antigenic modulation of the TL antigen by specific antibody involved in active metabolic process since it was blocked in cells exposed to actinomycin D. Thus, what might be considered a serologic phenomenon at the cell surface really represents the expression of cell metabolism and is susceptible to inhibition by drugs.

Dr. Weiss, although feeling immunologically exhausted by this time, urged renewed focusing on the activity of the host. Chemotherapy of infectious diseases is generally most successful when the host is able to contribute actively to his defense, and the limited but impressive successes of chemotherapy with some tumors might well be extended by elevating the inherent resistance mechanisms of the host. If one could, by immunologic means, bring a majority of the cells of a neoplastic mass into a resting state, then synchrony of regrowth might be facilitated by removal of the inhibiting immunologic parameter, and the entire population of tumor cells might then become sensitive to chemotherapeutic agents.

Dr. Mandel concluded the Conference Discussion on a note of thanks to all of the participants, the organizers, and the sponsors. The meeting had been informative to all participants, and numerous valuable ideas were presented which, hopefully, will stimulate further research. The conference and the extensive discussion provided a comprehensive general view of the current status of cancer chemotherapy. The close personal contacts between the various groups, representing chemotherapy, immunology, and clinical oncology have furthered mutual understanding and, simultaneously, have outlined more clearly the frontiers of our current knowledge and ignorance.
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