Formal Discussion: Future Prospects in Leukemia Chemotherapy[1]

EDWARD J. MODEST

The Children's Cancer Research Foundation, and the Department of Pathology, Harvard Medical School at The Children's Hospital, Boston, Massachusetts

In his characteristic way, Dr. Bergel has covered his difficult subject in an excellent manner and with a bold effort to outline new and hopeful approaches to the therapy of the leukemias. In fact, he has raised so many interesting points that I am in danger of taking more time for the discussion than he has taken for the presentation of his lecture. My only recourse is to be brief.

Dr. Bergel has outlined the fundamental problems in the treatment of the leukemias, including the lack of information on the etiology of human leukemia and the lack of a qualitatively different biochemical parameter of the leukemic cell. His general conclusion is that future research in chemotherapy ought to be directed to larger molecules, in particular to homeostatic regulators designed to "correct" the leukemic process. One can only applaud his many suggestions in this direction, although the road will not be easy because of the difficulty of working out and explaining the fundamental chemistry of many of the therapeutic agents and mechanisms proposed. For example, administration of macromolecules introduces enhanced problems of pharmacologic distribution, cell membrane penetration, and antigenicity. On the other hand, the real progress achieved to date with anti-leukemic agents of relatively low molecular weight and the importance of further work in this area should not be overlooked.

The investigation by Harrap and Speed (7) on the relationship of disulfide and thiol levels in the blood cells of patients with chronic myeloid leukemia to the state of the disease and its therapy is of great interest and could conceivably lead to the development of new agents or to information on the etiology of leukemia.

One of Dr. Bergel's suggestions is to work on hormone analogs, including those carrying a therapeutic residue, following a better understanding of the mechanism of action and resistance. We have already begun a program of synthesis of steroidal pyrimidines containing the growth-inhibitory 2,4-diaminopyrimidine nucleus (11) on the basis of our convenient synthesis of 2,4-diaminopyrimidine derivatives from ketones and dicyandiamide (9); a number of such compounds have been prepared. We are particularly interested in incorporation of the 2,4-diaminopyrimido moiety into steroid and carcinogenic hydrocarbon molecules by employment of our reaction method. For example, the direct synthesis of a 2,4-diaminopyrimido analog of cholesterol (Chart 1) has been effected by reaction of dicyandiamide with the keto-steroid cholestan-3-one. We are hopeful that steroidal pyrimidines of this kind will show antimetabolite properties, pharmacologically influenced by the steroid or transport part of the molecule.

Now I should like to discuss briefly 2 areas for future research on which Dr. Bergel did not elaborate. One of these is the potential of metastasizing experimental tumor systems for the detection of chemotherapeutic agents. Dr. Bergel has pointed out the imperfect correlation between the leukemias in man and the transplantable leukemias in animal systems. For example, the anti-leukemic agent 6-mercaptopurine was discovered originally to be a moderately effective inhibitor of Sarcoma 180 in the mouse (4). The application of metastasizing tumors in rodents may be of promise as a screening tool for new agents. Handler *et al.* (6) have been working on such systems and have found 1 of our compounds, 4,6-diamino-1-(3,4-dichlorophenyl)-2,2-dimethyl-1,2-dihydro-*s*-triazine naphthalene-1,5-disulfonate (D54·naponate) (Chart 2), to be at least as effective against the primary Lewis T-241 pleomorphic-cell sarcoma in C57BL/6 mice as many standard antitumor agents and superior in effect to any other compound studied against the pulmonary metastases (5) (Table 1). D54·naponate was designed as a repository salt form of the biologically active dihydrotriazine, with increased retention time *in vivo*. Because of its promising activity in experimental tumor systems, including rodent leukemias, D54·naponate is scheduled for clinical trial at the Children's Cancer Research Foundation in the near future.

Another highly speculative area is the apparent but poorly understood correlation between anti-leukemic activity and suppression of the immune response. In this connection I should point out that Hitchings and Elion (8) have prepared a useful review on the chemical suppression of the immune response. The variety of agents with temporary effectiveness against the leukemias (purine and pyrimidine analogs, folic acid antagonists, antibiotics, plant alkaloids, alkylating agents, and steroid hormones) indicates that compounds with obviously widely differing modes of action all produce what seems to be the same end reaction, namely, temporary remission. The only differences are the time of onset of the remission, the percentage response, the length of the remission, and the time of appearance of and the character of the resistance that invariably develops. It is, of course, possible that the anti-leukemic agents inhibit both the immune response and the leukemic process by direct cytotoxic action.

[1] Studies referred to in this paper were supported in part by research grants (CY3335 and C6516) from the National Cancer Institute, NIH, USPHS.

However, an explanation of the remissions in leukemia produced by these heterogeneous agents in terms of their immunosuppressive properties is an intriguing possibility, although a remote one at the moment.

One such possibility, based on the recent work of Adams (1–3) and developed in collaboration with him, is that anti-leukemic agents may act by blocking an immunologic enhancement mechanism on which the leukemic cell depends for survival in the host. This possibility is predicated upon the antigenicity of the leukemic cell in the host, and as Dr. Bergel has indicated, the evidence to date is uncertain. The conditions for survival of the leukemic cell by the enhancement phenomenon are attained if one assumes (*a*) a genetic mutation leading to the leukemic cell; (*b*) the presence of weakly antigenic sites on the leukemic cell embodying the "foreign" protein formed by mutation; (*c*) sufficiently weak antigenicity of these antigenic sites to preclude inactivation by humoral antibody; and (*d*) protection of these antigenic sites against cellular antibody by combination with humoral antibody. Then, if the immunosuppressive/anti-leukemic agent blocks humoral antibody production in preference to cellular antibody production, perhaps through interference with the protein synthetic mechanism, the leukemic cell is

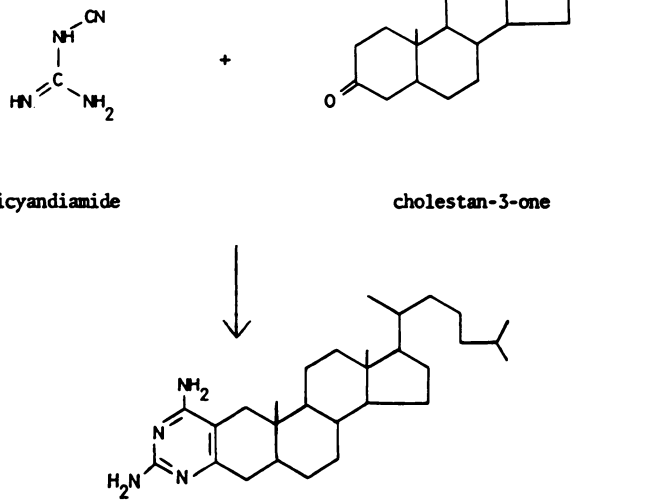

CHART 1.—Synthesis and chemical structure of 2′,6′-diamino-cholest-2-eno[3,2-*d*]pyrimidine, a steroidal pyrimidine.

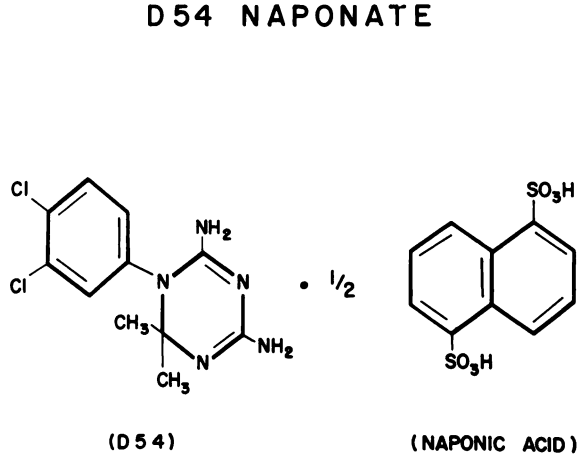

CHART 2.—Chemical structure of 4,6-diamino-1-(3,4-dichlorophenyl)-2,2-dimethyl-1,2-dihydro-*s*-triazine naphthalene-1,5-disulfonate (D54·naponate).

TABLE 1

CHEMOTHERAPY STUDIES ON PRIMARY TUMOR GRAFTS AND METASTASES OF LEWIS T-241 SARCOMA IN C57BL/6 MICE[a]

Compound	Dose, mg/kg/day × 7 (i.p.)	Primary tumor	Metastases with primary tumor intact	Metastases with primary tumor amputated
D54·naponate	100	2+	Moderate inhibition	Moderate inhibition
	80	2+	Slight inhibition	Slight inhibition
	50	2+	–	–
	40	2+	–	–
Actinomycin D	0.12	2+	Slight inhibition	Slight inhibition
	0.08	1+	–	–
	0.0625	1+	–	–
	0.05	–	–	–
Mitomycin C	2.0	2+	–	–
	1.0	2+	–	–
	0.5	2+	–	–
Methotrexate	10	2+	–	–
	5	1+	–	–
	2.5	–	–	–
5-Fluorodeoxyuridine	100–25	–	–	–
1-Methyl-1-nitrosourea	25–5	–	–	–
Sarcolysin	10–2.5	–	–	–
ThioTEPA[b]	5–1.25	–	–	–

[a] Evaluation of tumor inhibition (% inhibition based upon change in volume of treated tumors compared with change in volume of control tumors): 2+, 76–100%; 1+, 51–75%; ±, 26–50%; –, 0–25%.

[b] ThioTEPA, *N*,*N*′,*N*″-triethylenethiophosphoramide.

no longer protected by humoral antibody and can be destroyed by the cellular response.

The substantial progress that has been made in the chemotherapy of human leukemia is remarkable in view of these serious limitations: the etiology of the disease is obscure, and no qualitative biochemical difference has yet been defined distinguishing leukemic cells from normal cells. I strongly support Dr. Bergel's opinion of the importance of studies on the mechanism of action of anti-leukemic compounds, both those presently in clinical use and known to be temporarily effective and new compounds as they are developed. Such studies may well lead to solutions of the problems of resistance, to the development of more selectively acting agents, and possibly to the delineation of a unique biochemical property of leukemic cells.

In closing, I should like to mention some additional areas of research that I think will be of significance in the future. Very little has been done in these areas with respect to the development of improved anti-leukemic agents.

1. Interesting results should come from a recasting of "classical" antimetabolite theory in terms of the newer concepts of feedback inhibition and enzyme repression.

2. A study of anti-leukemic agents in the light of cellular regulatory mechanisms should be worthwhile. As anti-leukemic agents are developed that specifically interfere with biologic regulatory mechanisms in a definable manner, the likelihood of controlling leukemia will be greatly enhanced.

3. The specific design of inhibitors capable of independent inhibition of the synthesis of deoxyribonucleic acid, ribonucleic acid (RNA), and protein is of the greatest importance. Our knowledge to accomplish these ends will undoubtedly be refined in the near future. To split hairs even further, it would be of the greatest interest to have available separate inhibitors of the various forms of RNA, such as transfer, messenger, and viral RNA.

Finally, I should like to make the philosophical observation that, following the thinking of C. Northcote Parkinson (10), one can derive a variant of Parkinson's Law applicable to research in general—namely, that the number of rationalistic approaches to the eventual solution of a research problem varies inversely with the existing state of knowledge of the problem. This generalization adequately explains the multiplicity of suggestions presented at this meeting directed to the etiology, biologic and biochemical characterization, and therapeutic control of the leukemias. A corollary is that under such circumstances no reasonable approach should be neglected or condemned as unworthy. I am sufficiently optimistic to hope that some of these approaches will be fruitful in the immediate future.

REFERENCES

1. Adams, R. A. Heightened Immunity and Susceptibility toward Cheek Pouch Heterografts of a Mouse Leukemia in Syrian Hamsters. Cancer Res., *23:* 1834–40, 1963.
2. ———. Special Property of the Cheek Pouch in Heightened Susceptibility to Heterografts of a Mouse Leukemia. *Ibid.*, *23:* 1841–46, 1963.
3. ———. Enhancing Agent in the Plasma of Syrian Hamsters Bearing a Heterotransplantable Murine Leukemia. Federation Proc., *22:* 314, 1963.
4. Clarke, D. A., Philips, F. S., Sternberg, S. S., and Stock, C. C. Effects of 6-Mercaptopurine and Analogs on Experimental Tumors. Ann. N. Y. Acad. Sci., *60:* 235–43, 1954.
5. Farber, S., Modest, E. J., Handler, A. H., and Maddock, C. L. Effects of D54 Naponate, A Repository Dihydrotriazine Salt, on Rodent Tumors. Proc. Am. Assoc. Cancer Res., *4:* 19, 1963.
6. Handler, A. H., Sarris, T. C., and Wills, C. Chemotherapy Studies on Primary Tumor Grafts and Metastases in Hamsters and Mice. Acta Unio Intern. Contra Cancrum, *20:* 176–79, 1964.
7. Harrap, K. R., and Speed, D. E. M. Some Biochemical Aspects of Leukaemias: The Appearance of a Soluble Disulphide in the Blood in Chronic Granulocytic Leukaemia. Brit. J. Cancer, *18:* 809–17, 1964.
8. Hitchings, G. H., and Elion, G. B. Chemical Suppression of the Immune Response. Pharmacol. Rev., *15:* 365–405, 1963.
9. Modest, E. J., Chatterjee, S., and Kangur, H. Pyrimidine Derivatives. III. A Novel Synthesis of 2,4-Diaminopyrimidines. J. Org. Chem., *27:* 2708–9, 1962.
10. Parkinson, C. Northcote. Parkinson's Law. Boston: Houghton Mifflin Company, 1957.
11. Sengupta, S. K., and Modest, E. J. 2,4-Diaminopyrimidines from Dicyandiamide. Synthesis of Steroidal Pyrimidines. Abstracts of Papers, 147th Meeting, Am. Chem. Soc., Philadelphia, Pennsylvania, April 9, 1964, p. 20-M.