Summary of Informal Discussions on the Status of Alkylating Agents in Current Chemotherapy

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Strong views were expressed by Dr. Burchenal, and supported by Dr. Holland, that further general synthesis, screening, and clinical testing of new alkylating agents were likely to be unrewarding and should be discouraged. Dr. Friedman pointed out that this restraint should not include the synthesis of specialized alkylating agents designed with a rationale which might exploit some known difference between tumor and normal cells.

Dr. Segaloff and Dr. Rundles agreed with Dr. Schmidt that more careful studies of the clinical utility of some of the known alkylating agents would be likely to be useful. In a comparative study begun five years ago, Dr. Rundles reported that Myleran was a poor agent for chronic lymphocytic leukemia but gave 65–70 per cent remissions with granulocytic leukemia, with excellent control for as long as 10 years. He also reported that HN2 was far superior to P32 in the control of granulocytic leukemia. Phenylalanine mustard also proved effective. Cytoxan was found to give some improvement in about 10 per cent of patients with bronchogenic carcinoma. Dr. Rundles also found somewhat more favorable response from mustard on carcinoma of the breast, with 40 per cent favorable responses.

Dr. Frei suggested that screening of new alkylating agents against phenylalanine-resistant Yoshida, Dunning, and Walker tumors might provide a useful basis for decision as to agents worthy of clinical trial.

Dr. Price pointed out that Dr. Ross's representation of alkylating agents as acting through carbonium ions was purely schematic for nitrogen mustards, since they in fact involve cyclic imonium ions as intermediates.

Dr. Warwick questioned whether or not this would apply also to aromatic nitrogen mustards.

Dr. Price pointed out that Dr. William J. Steele (W. J. Steele and C. C. Price, IV Internatl. Cong. Biochem., Moscow, p. 398, 1961; W. J. Steele, Proc. Am. Assoc. Cancer Res., 3:364, 1962; Biochem. Pharmacol., in press) has shown that one of the important effects of HN2 on Ehrlich ascites cells at therapeutic dose levels is to cause considerable cross-linking of protein to DNA. The protein content of DNA was increased from 1–2 per cent levels to 5–10 per cent by such treatment, but not by the monofunctional alkylating agent, hemisulfur mustard. By use of C14-labeled HN2 it was possible to show that there were about two bound HN2 molecules per 50,000 tide units in the DNA. This suggests that the bound HN2 is mainly utilized to cross-link DNA to protein. By alkylation with HN2-C14 followed by ultracentrifugation in cesium chloride gradient, Dr. Robert Rutman and Dr. E. H. L. Chun have shown that the main part of the C14 appears in a spur band slightly less dense than the DNA, also in agreement with the concept that bound HN2 is in a fraction containing some protein.

Dr. Price also mentioned that Dr. Rutman has shown that alkylation of Ehrlich ascites cells at chemotherapeutic levels produces an 80–85 per cent inhibition of the incorporation of tritium-labeled thymidine into DNA. He has also found that alkylation does not alter the "melting point" of Ehrlich DNA but does substantially increase the "renaturation" of "melted" DNA, another observation supporting the concept of cross-linking action of HN2 and not readily explained by the guanine-deletion hypothesis.

Dr. Reich raised a question with Dr. Warwick as to how the guanine-deletion hypothesis of Lawley could explain the observations indicating cross-linking has occurred.

Dr. Warwick expressed the view that deguanylation of alkylated moieties would occur in the cell, whereas Dr. Price indicated that this must still be considered only hypothesis.

Dr. Heidelberger pointed out that Dr. Kendrick Smith in Henry Kaplan's laboratory at Stanford has also presented evidence for cross-linking of DNA to protein by exposure to X-radiation.

Dr. Kensler asked whether the hypothesis that mustard-resistant cells contained excessive...
free sulfhydryl groups in the plasma would not imply accumulation of labeled alkylating agents in supernatant. DR. WHEELER indicated such studies were in progress.

DR. FRIEDMAN made the following comments on the mechanism of action of Cytoxan (cyclophosphamide):

"The mechanism of action of Cytoxan, as Dr. Wheeler indicated, is far from clearly established. This is one of the cases in which a compound had been designed with a specific type of biochemical rationale in mind. This compound was designed with the hope or expectation that the level of certain hydrolytic enzymes of the type that would activate it would be higher in malignant cells than they are in normal tissues.

"There has been some confusion about the nature of these enzymes, the phosphamidases, and question as to whether, in fact, they exist as discrete enzymes distinct from the phosphatases. We have evidence that they do, although it is not at all certain now that they are actually involved in the mechanism of action of Cytoxan.

"As regards the metabolic fate of Cytoxan, we have done some work on this with Dr. Foley at the Children’s Cancer Research Foundation, in which we found indications of metabolic change. As you know, Cytoxan is chemically inert in vitro and no evidence whatsoever to date to indicate that metabolism of Cytoxan results in the liberation of norHN2 or, in fact, of any other alkylating agent. If Cytoxan is heated in buffers at pH values in the range of 3–10, and then in strong acid in order to liberate unchanged norHN2, very little nonHN2 can be found even after short periods of incubation; it disappears with remarkable rapidity. In other words, it appears that in buffers other than strong acid even the chemical hydrolysis of Cytoxan does not involve the release of norHN2.

"One other assumption that is being made here is that this active metabolite of Cytoxan in the liver is the component that ultimately produces inhibition of tumors remote from the liver. This is not at all certain, of course, but apart from this assumption what we come to is that there is no evidence whatsoever to date to indicate that metabolism of Cytoxan results in the liberation of norHN2 or, in fact, of any other alkylating agent.

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followed up the work we did on the activation of Cytoxan in vitro with liver extract. They have come to the conclusion, first of all, that this activation doesn’t take place in the soluble components. Activation occurs, they believe, in the mitochondrial fraction. They further find that activation will not take place in the absence of oxygen, and have concluded that it is an oxidative process. They also state that they find no evidence whatsoever that the process of activation involves the release of norHN².

“...I find the foregoing interesting in relation to the conclusions that have been arrived at concerning the mode of action of Cytoxan and its cross-resistance with alkylating agents. I wonder, if Cytoxan had not been labeled as an alkylating agent to begin with, whether the same conclusion would have been reached as regards its common mechanism with the alkylating agents, or, in fact, whether it would have been possible to distinguish it from some of the antimetabolites as regards its mode of action.”
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