Summary of Informal Discussion on Biochemical Resistance to Chemotherapeutic Agents

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The general background material for the discussions of resistance to chemotherapeutic agents was outlined in the presentation by DR. HUTCHISON. The scope of the current problem was indicated by the compilation of anticancer drug-resistant experimental biologic systems reported during the past 2 years (1962–1964). Data on the effects of many chemotherapeutic agents on the refractory systems were also considered. The multiplicity of mechanisms for resistance to a given agent was emphasized; it was indicated that the many facets of the problem of resistance need not be a disadvantage but, instead, a help in better understanding the biochemical constitution of the leukemic cell.

DR. BROCKMAN reviewed the recent literature on mechanisms of resistance to purine antagonists in a variety of biologic systems. The most common mechanism for resistance was the loss of nucleotide-forming capacity. The purine nucleotide pyrophosphorylases of a variety of materials were classified into 3 groups based on substrate specificity and correlated with patterns of cross-resistance. Further discussion was concerned with selective pressures and their effects on the resultant resistant populations. Several observations were cited in respect to the mode of action of 6-mercaptopurine (6-MP),[1] and the question of the primary site of action of 6-MP was raised. It was suggested that the feedback control site, i.e., synthesis of phosphoribosylamine, is the site 1st inhibited by 6-MP-ribonucleotide. That 6-MP-ribonucleotide is a feedback inhibitor of *de novo* purine biosynthesis was also confirmed by the lack of such inhibition in extracts of a neoplasm known to be deficient in inosinic acid-guanylic acid (IMP-GMP) pyrophosphorylase.

The studies with experimental neoplasms prompted DR. DAVIDSON to ask if the loss of IMP-GMP pyrophosphorylase were the major mechasnim of resistance to 6-MP in human leukemia. Of his 6 patients with 6-MP-resistant leukemia, only 1 showed a decreased capacity for the formation of 6-MP-ribonuleotide. The cell extracts of all the patients, both 6-MP-sensitive and 6-MP-resistant, showed an elevated adenylic acid (AMP) pyrophosphorylase activity. Dr. Davidson enumerated the problems associated with the use of human materials in experimentation. He suggested that some of the difficulties inherent in the use of human materials could be alleviated if one could grow leukemic cells in a continuous culture system.

DR. HITCHINGS added to the point of selective pressures, discussed by Dr. Brockman, in the evolution of resistant populations by citing work from his laboratory on the selection of pyrimethamine-resistant mutants of *Lactobacillus casei* from media containing a purine and a thymine, either one alone, or neither. Mutants selected from media containing either or both nucleic acid derivatives were auxotropic, whereas in the absence of purine and/or thymine the mutants were prototropic. Thus the general nutritional state of the system tends to influence the resistant population. This is a situation that should be recognized in considering the problems associated with resistance in human leukemia.

Dr. Hitchings also indicated that xanthylic acid pyrophosphorylase must be present in the mouse; he recently observed that by using a xanthine oxidase inhibitor (4-hydroxypyrazolo-[3,4-*d*]pyrimidine) in combination with xanthine the latter was incorporated into nucleic acids. In view of Dr. Davidson's results, the high levels of AMP pyrophosphorylase in the leukemic cell makes it obvious that a way to treat leukemia is with an adenine antagonist; why doesn't it work?

DR. BURCHENAL indicated that 2,6-diaminopurine had been used several years ago and a good remission was obtained in 1 patient. He also stressed the unpleasant toxic effects of 2,6-diaminopurine on the patient.

DR. LEPAGE speculated that there may be a basis for drugs to discriminate between normal and neoplastic cells; for example, cells may lose a certain metabolic facility during the cancerogenic change. In his laboratory numerous studies have been conducted on mouse neoplasms resistant to thioguanine (TG), and 3 of the experimental neoplasms exhibit a biochemical alteration that has not been mentioned here. They all have an adequate capacity to convert thioguanine to its ribonucleotide but cannot convert this to the appropriate deoxyribonucleotide. With the expectation that the cells would have the appropriate kinase, Dr. L. Goodman synthesized 2′-deoxythioguanosine (TGDR). In the 3 thioguanine-resistant neoplasms it was found that TGDR was converted to a nucleotide and produced tumor inhibition.

DR. HANDSCHUMACHER mentioned the work of the Southern Research Institute group on bis-thioinosine-5′,5‴-phosphate. This compound had been suggested as one to circumvent the resistance mechanism in cells that have lost a specific nucleotide pyrophosphorylase. In an

[1] The following abbreviations are used: 6-MP, 6-mercaptopurine; IMP, inosine monophosphate; GMP, guanosine monophosphate; AMP, adenosine monophosphate; TG, thioguanine; TGDR, deoxythioguanosine; and AZUR, azauridine.

attempt to explore the inhibitory capacity of a similar pyrimidine analog, Dr. Glenn A. Fischer submitted a line of L5178Y resistant to 6-azauridine and deficient in uridine kinase to the potential growth-inhibitory effects of bis-6-azauridine-5′,5‴-phosphate. The uridine analog was synthesized by Dr. John Montgomery, and the 50% inhibitory concentration of bis-6-azauridine-5′,5‴-phosphate was compared with 6-azauridine and 6-azauridine-5′-phosphate. The inhibitory concentrations were as follows for L5178Y and L5178Y/6-AZUR, respectively: 6-azauridine, 1×10^{-7} M and 2×10^{-6} M; 6-azauridine-5′-phosphate, 2×10^{-7} M and 3×10^{-6} M; bis-6-azauridine-5′,5‴phosphate, 4×10^{-7} M and 6×10^{-6} M. The bis-nucleotide either was not taken up by these L5178Y cells or was not cleaved to an active inhibitor. In reply to a query from DR. FRIEDKIN, it was pointed out that the 6-azauridine-resistant line was only partially deficient in kinase activity, which accounts for the 20-fold increase in resistance.

Dr. Handschumacher and Dr. Fischer have indicated interest in determining the specificity of the pyrimidine nucleoside kinases. They have selected clones of L5178Y resistant to low levels of 6-azauridine, 6-azacytidine, cytosine arabinoside, and 5-fluorodeoxyuridine, and the results of some preliminary testing suggest that lowered kinase activity may account for some cases of cross-resistance but not necessarily all cases. In the mutants examined there was no indication of an altered enzyme.

DR. WERKHEISER discussed in detail the known limitations of the therapeutic effectiveness of amethopterin. These limitations are primarily toxicity and resistance (natural or acquired). It was strongly suggested that both of these obstacles could be overcome by the design of new anti-folics in association with the clarification of the mechanism of resistance in human leukemia.

DR. BERTINO described his current studies on the folate antagonists in patients with acute leukemia. The presentation was concerned with dihydrofolate reductase activity, the responsiveness of the disease to methotrexate, and the current status of studies on resistance. To date none of the mechanisms of resistance as exemplified by the experimental systems has been observed in human leukemia. The reason is that until now it has not been feasible to carry out the same kind of sophisticated studies with human materials.

DR. FRIEDKIN discussed attempts to achieve a novel situation of lethal synthesis by cells resistant to amethopterin and possessing a high dihydrofolate reductase activity. In order for this lethal synthesis to work, the analog must be a good substrate for reductase and the product of the reaction must inhibit thymidylate synthetase or some other tetrahydrofolate-requiring enzyme system. Friedkin and his associates employed a 2-enzyme screen 1 to test the effectiveness of several folic acid derivatives as a substrate for reductase and 2 to test the inhibitory properties of the tetrahydro-derivatives against thymidylate synthetase. Homofolic acid (Goodman *et al.*, J. Am. Chem. Soc., *86:* 368, 1964) is the most promising compound thus far. Dihydrohomofolate is an excellent substrate for a mouse leukemia reductase, and the tetrahydro-derivative inhibits thymidylate synthetase at 10^{-6} M. Dr. Roy Kisliuk has found that the growth of *Streptococcus faecalis* is markedly inhibited by tetrahydrohomofolate.

DR. HARRINGTON asked Dr. Bertino if there had been confirmation of the studies of Dr. Wallerstein and his associates on the alternate treatment of leukemic mice with a folate analog and a purine and then 6-MP and folic acid, which supposedly delays the emergence of resistant cells and thereby prolongs the life of leukemic mice. DR. BERTINO indicated that Dr. Wallerstein has similar studies in progress in some cases of human leukemia; the preliminary indications, though not definitive, are encouraging.

The results presented on the many aspects of resistance were encouraging and do indicate that the elucidation of the "resistance phenomena" in human leukemia will soon be achieved. In general there was agreement on the seriousness and complexity of the problems of drug resistance related to human leukemia. There was optimism since it now may be possible to take advantage of the obstacle of resistance for the design of new drugs. Also it may be possible to indicate a more effective drug as a result of a known altered metabolic pathway in a given resistant population.