Formal Discussion: Some Reflections on the Basis of Drug Failures

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A misplaced comma in a computer program can result in the premature and fiery end of a costly space rocket experiment. In cancer research the commas are usually in place but the programs seem to be wrong!

Only ephemeral successes have been achieved by use of chemotherapeutic agents for the control of acute leukemia. The disappointments have spread out over the years despite the introduction of many sophisticated approaches. Whatever effect (usually a toxic one) an anticancer agent may have on the patient, all new drugs seem to have one pharmacologic property in common: a psychotomimetic effect on the innovator who, either by chance or by clever design, has introduced the "curative" agent. The hallucinogen magnifies the minor drug successes to an extraordinary degree while the major drug failures are blotted out. Meanwhile the experimental animals go on dying and, alas, the patients as well.

It is the sharp hurt of drug failure that has led able investigators such as Dr. Skipper and members of his group to consider in depth the kinetics of leukemic cell behavior. What is there about the population explosion of leukemic cells that makes drug control so promising at first yet so unsuccessful at last?

The implications of exponential growth of leukemic cells, although generally understood, are too often forgotten. If 1 leukemic cell survives, whether it be because of inadequate drug dosage, because of protection from the drug in protected areas of the brain (8, 12), or because of biochemical alteration which confers drug resistance (2, 9), the inexorable doubling process leads to a logarithmic increase that is eventually lethal to the host.

Goldin *et al.* (6) concluded some years ago that the number of leukemic cells in the host can influence the apparent effectiveness of drug treatment. The prolongation of the life span of the host becomes a function of the time it takes for the drug-sensitive leukemic cell population to reach a lethal number. Skipper *et al.* (11) have amassed a considerable amount of data in support of the lethal number concept and now agree with Goldin that in the transplantable experimental leukemias the cause of leukemic death may be due to the fractional survival of drug-sensitive cells.

I am uncertain whether Dr. Skipper believes that the death of a leukemic patient can also be attributed to the continued proliferation of drug-sensitive cells because of inadequate treatment. I can understand how meningeal leukemia might be due to the proliferation of drug-sensitive cells protected by the blood-brain barrier and how meningeal leukemia might reseed peripheral tissues during periods of low drug levels; however, I would argue that the emergence of drug-resistant cells can be an important element of lethality in human leukemia.

Since my own interests have been mainly in the field of folic acid metabolism, I prefer to limit this discussion to problems associated with the use of folate antagonists. Much has been written about the pharmacologic effects and mode of action of the powerful anti-leukemic analogs of folate (1, 13), yet we have no clear understanding of the basis of eventual drug failure in man.

Does the chemotherapeutic action of amethopterin in a leukemic animal or man depend on the outright killing of a certain fraction of drug-sensitive cells or on the prolongation of the generation time of all drug-sensitive cells? Neither Dr. Skipper's studies nor the collaborative efforts of Dr. Goldin's group and our laboratory (4) provide a clear answer to this question.

Dr. Walter Hughes, to whom we owe so much for introducing the use of thymidine labeled with tritium, has suggested (7) that iododeoxyuridine (10) labeled with a γ-emitting isotope of iodine might provide a means of evaluating deoxyribonucleic acid (DNA) synthesis and cell death in the intact leukemic mammal. Iododeoxyuridine labeled with ^{131}I is poorly utilized as a precursor of DNA because of the deiodination that occurs shortly after injection into an animal. However, once incorporated into DNA, iodine linked to pyrimidine is not lost as long as the cell remains intact. It should be stressed that despite the rapid elimination of iodine from freshly injected iododeoxyuridine, enough labeled deoxynucleoside remains to serve as a very useful tracer of DNA synthesis. If a cell containing iodinated DNA dies, breakdown of DNA results in the eventual release of iododeoxyuridine, which in turn gives rise to inorganic iodide-131.

Hughes *et al.* (7) have taken advantage of the poor initial utilization of iododeoxyuridine to study cell turnover since rapid deiodination limits the reutilization of radioactivity from dying cells. For example, cell death can be observed in the intestine within 36 hr after labeling with iododeoxyuridine. The kinetics of cell death can be studied with a combination of ^{125}I-iodide and ^{131}I-deoxyuridine. Any increase in the ratio of iodide-131 to iodide-125 after incorporation of iododeoxyuridine into DNA becomes a measure of cell death.

The double tracer technic of Hughes *et al.* (7) might therefore prove to be extremely useful in distinguishing between the killing effects and generation-prolonging effects of amethopterin in experimental leukemias.

How could an accurate assessment be made of the number

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of amethopterin-resistant cells in a mixed population of leukemic cells? This problem as it applies to man has been considered especially by Bertino (1).

In our studies with Dr. Goldin of mixed populations of sensitive and resistant cells in leukemic mice (4), we concluded that the assay of dihydrofolate reductase might provide an accurate way of following the emergence of amethopterin-resistant cells. Of great value for studies with human tissues would be a histochemical procedure involving the use of a folate derivative that upon reduction via dihydrofolate reductase would label the drug-resistant cell either by fluorescence or by color. As yet the proper substrate for this purpose is not available.

Another approach might depend upon radioautography with a combination of labeled thymidine and deoxyuridine. Aminopterin blocks the short period utilization of radioactive deoxyuridine for biosynthesis of DNA, but not that of radioactive thymidine (5). The aminopterin-resistant leukemic cell would presumably incorporate both deoxynucleosides in the presence of the drug.

Radioautographic studies with tritium-labeled amethopterin patterned after the work of Fischer *et al.* (3) not only should provide valuable information about the increased binding of the radioactive anti-folate in individual resistant human leukemic cells characterized by high dihydrofolate reductase content but also should reveal those cells in a population that have become resistant because of a decreased transport of the drug.

In conclusion, I would like to support wholeheartedly Dr. Skipper's contention that the attempt to analyze the kinetics of leukemic cell behavior is not an idle academic exercise. The existence of changing cell populations is fundamental to the evolution of all organized groups of differentiated cells. The knowledge of how a drug can influence a population of leukemic cells may help us to understand eventually the control processes inherent in maintaining the extraordinary situation we call normalcy.

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