dose of ara-C was not determined.

Thus, deaminase activity in leukemic cells is not likely to be a mechanism of resistance to ara-C, and THU does not offer a therapeutic advantage in overcoming cellular ara-C resistance. However, because it is deaminated in the liver in humans, THU may be useful to produce a higher ara-C plasma concentration, an effect that could be achieved by increasing the ara-C dose. Dosage of ara-C could be individualized by use of an immunoassay for rapid measurement of plasma levels. It seems unlikely that there will be any differences in toxicity or therapeutic effect if ara-C is given by infusion and similar levels are maintained by either increasing the ara-C dose or using THU.

REFERENCES

1. Coleman, C. N., Stoller, R. G., Drake, J. C., and Chabner, B. A. Deoxycytidine kinase: properties of the enzyme from human leukaemic granulocytes. Blood, *46:* 791–803, 1975.
2. Harris, A. L., and Grahame-Smith, D. G. Variation in sensitivity of DNA synthesis to Ara-C in acute myeloid leukaemia. Br. J. Haematol., *45:* 371–379, 1980.
3. Harris, A. L., and Grahame-Smith, D. G. Cytosine arabinoside deamination in human leukaemic myeloblasts and resistance to cytosine arabinoside therapy. Clin. Sci. (Oxf.), in press, 1980.
4. Harris, A. L., Potter, C., Bunch, C., Boutagy, J., Harvey, D. J., and Grahame-Smith, D. G. Pharmacokinetics of cytosine arabinoside in patients with acute myeloid leukaemia. Br. J. Clin. Pharmacol., *8:* 219–227, 1979.
5. Henderson, J. F, Brox, L. W., Fraser, J. H., Lomax, C. A., McCoy E. E., Snyder, F., and Zomobr, G. Models and methods for biochemical studies of resistance in man. *In:* Pharmacological Basis Of Cancer Chemotherapy, pp. 663–680. Baltimore: The William & Wilkins, Co., 1975.
6. Ho, D. H. W, Carter, C. J., Brown, N. S., Hester, J., McCredie, K., Benjamin, R. S., Freireich, E. J, and Bodey, G. P. Effects of tetrahydrouridine on the uptake and metabolism of 1-β-D-arabinofuranosylcytosine in human normal and leukemic cells. Cancer Res., 40: 2441–2446, 1980.
7. Kreis, W., Hession, C., Soricelli, A., and Scully, K. Combination of tetrahydrouridine and cytosine arabinoside in mouse tumours. Cancer Treat. Rep., *61:* 1355–1364, 1977.

Adrian L. Harris
The Royal Marsden Hospital
London and Surrey
Fulham Road
London SW3 6JJ
England

Correspondence re: D. H. W. Ho *et al.*, Cancer Res., *40:* 2441–2446, 1980 (cont'd.).

After carefully reading Dr. Harris' letter and our paper (5), we find for the following reasons that neither Dr. Harris nor we have presented data dealing with the mechanism of resistance to ara-C[1] in human leukemic myeloblasts. (*a*) Our work was strictly an *in vitro* cell uptake study which cannot possibly be used to draw a conclusion regarding patient response *in vivo*. And (*b*) Dr. Harris has not identified responses to ara-C treatment in his patient population, nor were the effects of THU on ara-CTP and ara-U formation in leukemic cells correlated with patient responses to ara-C. Therefore, we would like to know how Dr. Harris derived his conclusion, "ara-C deamination in human leukemic myeloblasts is not a mechanism of resistance to ara-C therapy." If he does have data to support his statement, it would indeed be an important although controversial finding, since Steuart and Burke (6) have demonstrated that "initial therapeutic responses to ara-C treatment in human leukemia are correlated with lower intracellular concentrations of deaminase than those present in non-responders. . . ."

In our studies, we did not identify the responses of the patients to ara-C treatment because those patients were on combination chemotherapy of ara-C with several other drugs which made the evaluation impossible. We made no conclusion about the response to ara-C therapy and its correlation to ara-C deamination. Nor did we make the conclusion that "deaminase activity in leukemic cells is likely to be a mechanism of resistance to ara-C and THU offers a therapeutic advantage in overcoming cellular ara-C resistance." In fact, our feelings on the mechanism of ara-C resistance are expressed in the following quote, "At present, the mechanism of clinical resistance to ara-C has not been defined with certainty" (Ref. 5, p. 2445, Paragraph 2, Line 1).

Dr. Harris found, in Paragraph 6, that "the increase in ara-CTP of human leukemic cells produced by THU was proportional to the change in ara-C concentration in the medium." His results support our findings also. Furthermore, his results substantiate our speculations that "THU may spare ara-C from degradation and permit target cells to be exposed to the agent" (Ref. 5, p. 2441, Paragraph 4, Line 17).

We have certainly made no conclusion that "deaminase exerts its effect by competing intracellularly with deoxycytidine kinase for ara-C." Our work was to show and to explain, in part, the *in vitro* differential THU effect on the formation of ara-CTP and ara-U among acute myelocytic leukemia, chronic myelocytic leukemia, and chronic lymphocytic leukemia cells (5).

Measuring the deaminase activity, we have indeed used 5 times the substrate concentration to measure the kinase activity. These concentrations were used because the reported K_m's for the deaminase and kinase activities of human leukemic cells have an approximate 4-fold difference (1, 4). Using ara-C as a substrate, the K_m for deaminase of human leukemic cells is 9×10^{-5} M (1) and for kinase is 25×10^{-6} M (4). Conventionally, it is necessary to measure enzyme activities under optimum conditions, *e.g.*, pH, buffer, cofactors, substrate concentration, etc. One could, of course, argue that these conditions are not the same as in intact cells and *in vivo*. In fact, the kinase reaction has always been measured with the addition of 10^{-3} M THU to prevent substrate consumption by the deaminase (3).

We totally agree with Dr. Harris that measuring actual products of ara-C metabolism in intact cells [as Dr. Harris, Chou *et al.* (2), and we (5) have done], rather than using the enzyme activity, may be more representative of the *in vivo* metabolism. However, measuring enzyme activities is not totally uninformative. Without this *in vitro* technique, we would not know that human liver is the major site of ara-C inactivation and why ara-C is rapidly deaminated to ara-U *in vivo*.

[1] The abbreviations used are: ara-C, 1-β-D-arabinofuranosylcytosine; THU, tetrahydrouridine; ara-CTP, 1-β-D-arabinofuranosylcytosine 5′-triphosphate; ara-U, 1-β-D-arabinofuranosyluracil.

Received March 16, 1981; accepted April 14, 1981.

We agree with Dr. Harris that THU may be useful in retaining a higher plasma ara-C concentration, an effect that could also be achieved by increasing the ara-C dose. However, a possibility still exists that if ara-C and THU are used as a combination therapy, THU will not only protect ara-C from deamination by the human liver, it may also inhibit ara-C deamination in the leukemic cells. Therefore, intact ara-C available in circulation may be transported to leukemic cells in which no further deamination would occur and perhaps more phosphorylation could take place (Dr. Harris; Refs. 2 and 5). The schedule and doses of ara-C and THU would have to be adjusted accordingly. Needless to say, further work is needed to substantiate or refute these speculations.

We hope we have answered Dr. Harris' questions and clarified his understanding of our work and results.

ACKNOWLEDGMENTS

I would like to express my gratitude to Drs. D. Farquhar, W. Kreis, and W. Plunkett for their helpful discussions and to S. Rose for her editorial assistance.

REFERENCES

1. Chabner, B. A., Johns, D. G., Coleman, C. N., Drake, J. C., and Evans, W. A. Purification and properties of cytidine deaminase from normal and leukemic granulocytes. J. Clin. Invest., *53:* 922–931, 1974.
2. Chou, T. C., Arlin, Z., Clarkson, B. D., and Philips, F. S. Metabolism of 1-β-D-arabinofuranosylcytosine in human leukemic cells. Cancer Res., *37:* 3561–3570, 1977.
3. Coleman, C. N., Johns, D. G., and Chabner, B. A. Studies on mechanism of resistance to cytosine arabinoside. Problems in the determination of related enzyme activities in leukemic cells. Ann. N. Y. Acad. Sci., *255:* 247–251, 1975.
4. Coleman, C. N., Stoller, R. G., Drake, J. C., and Chabner, B. A. Deoxycytidine kinase: properties of the enzyme from human leukemic granulocytes. Blood, *46:* 791–806, 1975.
5. Ho, D. H. W., Carter, C. J., Brown, N. S., Hester, J., McCredie, K., Benjamin, R. S., Freireich, E. J, and Bodey, G. P. Effects of tetrahydrouridine on the uptake and metabolism of 1-β-D-arabinofuranosylcytosine in human normal and leukemic cells. Cancer Res., *40*: 2441–2446, 1980.
6. Steuart, C. D., and Burke, P. J. Cytosine deaminase and the development of resistance to arabinosylcytosine. Nature (Lond.), *233:* 109–110, 1971.

Dah Hsi Ho
Developmental Therapeutics, CP-1030
M.D. Anderson Hospital and Tumor Institute
Texas Medical Center
Houston, Texas 77030