superior survival in patients who have a shorter interval from diagnosis to transplant. The authors point out that the prognostic implication of the interval from diagnosis to transplant is of particular interest since it is under the control of the patient and the physician.

An additional factor under the control of the physician may be of comparable importance. In their original report, Thomas et al indicated that the poorer survival of patients with a long interval from diagnosis to transplant might result from either the disease or its treatment. Patients with a long interval from diagnosis to transplant, in whom information about chemotherapy received before transplant was available, had received busulfan. Many of the patients with a short interval had not received busulfan. Although analysis of patients who received busulfan showed a poorer survival associated with a long interval, this might have been a consequence of cumulative busulfan toxicity. The incidence of lethal interstitial pneumonia was higher in individuals who received busulfan. Busulfan has a number of serious potential side effects, including the development of pulmonary fibrosis, prolonged myelosuppression, hypogamandism, or an Addisonian-like state. In contrast, hydroxyurea is not associated with these side effects. In most individuals with CML, the use of busulfan has no significant advantages over hydroxyurea and may jeopardize future marrow transplantation.

It appears that marrow transplantation will be performed in increasing numbers of individuals with CML, including perhaps many who do not have human leukocyte antigen-identical sibling donors. Physicians caring for patients with chronic myelogenous leukemia should consider the potentially adverse effect of busulfan on survival after marrow transplantation. The excellent survival achieved with the regimen described by Thomas and Clift should focus additional attention on the impact of pretransplant management on survival after marrow transplantation for patients with CML as well as other disorders.

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REFERENCES


BIOTINYLATED ERYTHROPOIETIN

To the Editor:

In a recent article by Wojchowski and Caslake, the utility of biotinylated recombinant human erythropoietin for measurement of receptor number and affinity was described. Our experience with biotinylated peptide hormones leads us to believe that they will undoubtedly be useful reagents for studying the corresponding receptors. However, this experience also leads me to question the application described. My major concern is in the use of these reagents for Scatchard analyses. Since the detection of signal with [125I]-streptavidin requires several washes (the number is not stated) after the incubation with biotinylated erythropoietin, it is clear that equilibrium conditions are not in effect since the degree of dissociation of the hormone to the receptor during the wash steps was not determined. This is not to be confused with the time required for equilibrium binding to be achieved. In addition, as the investigators correctly state, the values for receptor affinity and number depend on the assumption that, on average, one [125I]-streptavidin binds per receptor. While they may be correct, the fact that streptavidin is tetravalent, and that each recombinant erythropoietin may have from 5 to 19 molecules of sialic acid (and therefore possibly many biotins), their assumption should be validated.

Second, there was no attempt to remove unreacted hormone from the biotinylated material before the binding analyses. If, as suggested, there was a 55% loss in biologic activity due to the biotinylation steps, then the use of a mixture of fully active nonbiotinylated material plus partially active biotinylated material will likely give underestimates of affinity and number of receptors. Third, determination of the activity of the biotinylated material relative to the native hormone is best done after determination of protein content by amino acid analysis. The units per milligram of protein can then be expressed and compared with the values given for the native hormone. This avoids the pitfalls of using relative concentrations adjusted for volume of elution from biotin blocked or unblocked streptavidin agarose columns.

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RESPONSE

Dr Newman makes the obvious theoretical point that receptor affinity and density are measured most accurately using a ligand which is directly radiolabeled, chemically uniform, and fully bioactive. However, as discussed in our publication, substantial to complete inactivation results from radiiodination of erythropoietin using either chloramides or Bolton-Hunter reagent. This circumstance, in part, provided impetus for our development of biotin-sialyl-erythropoietin as a bioactive probe for receptor. Based on three assumptions, Newman questions the value of assessing the binding properties of this ligand. Newman first assumes that equilib-
Biotinylated erythropoietin [letter; comment]

W Newman