To the Editor:

Gordon and Hubbard\(^1\) designated some cases as acute myelomonocytic leukemia because of leukocytes that stained with both the AS-D-chloracetate esterase and the \(\alpha\)-naphthol acetate esterase (NSE) reactions. The dual reactivity is real; however, there is an alternative to the interpretation that the cell is a monocyte or monocyte hybrid (myelomonocyte). Recent observations that I made suggest that the cell in Fig. 4 of ref. \(^1\) is a neutrophil myelocyte.

While studying a patient with systemic mastocytosis who progressed through the phase of an "unclassified" myeloproliferative disorder into acute myeloblastic leukemia, I noted a marked discrepancy between cytochemical reactivities and the morphology of the blood cells. The studies reported here were made during the year before he developed acute leukemia, at which time his peripheral leukocyte counts were elevated (20,000–35,000/\(\mu\)l, with 55%–60% neutrophils, 15%–16% monocytes). The bone marrow showed patchy dense fibrosis characteristic of mastocytosis, with the intervening marrow space markedly hypercellular. The neutrophilic series was predominant (65%–70% of nucleated cells), marrow monocytes were inconspicuous with Wright stain (< 4%), and myeloblasts were normal in number. Electron microscopy of the bone marrow showed neutrophilic predominance. Cytochemical studies with Sudan black B, peroxidase, and PAS stains\(^2\) were all consistent with a neutrophilic-type differentiation in 65%–70% of nucleated marrow cells. The AS-D-chloracetate esterase stain\(^3\) showed 55%–60% of marrow nucleated cells with significant reactivity. Stains for NSE\(^4\) using either \(\alpha\)-naphthol acetate or butyrate as the substrate showed about 37% of the nucleated marrow cells to be strongly positive and an additional 22%–25% to be moderately positive. Less than 40% of cells showed negative or trace reactivity or contained the coarse focal positivity characteristic of some lymphocytes. All of the strongly and moderately positive cells showed marked sensitivity to fluoride inhibition. These results have been confirmed by repeated testing on both nonanticoagulated marrow smears (directs) and EDTA-anticoagulated smears of the marrow M-E layer (concentrates).

Because of the large population of NSE-positive cells, a pattern of reactivity in the neutrophilic series was obvious, with the greatest degree of positivity occurring in the promyelocyte-myelocyte stage and an abrupt decreased positivity in the very earliest and the later developmental stages.

With the experience gained from this case, I reviewed two other cases of hematopoietic dysplasia where we had noted a larger number of NSE-positive cells than the number expected from examination of the Wright stain, as well as another case of systemic mastocytosis associated with a myeloproliferative disorder. In each of these cases, it was possible to separate morphologically a group of cells similar in all respects to the neutrophil precursors previously identified and to trace NSE activity into smaller numbers of more mature forms.

Demonstration by Kass et al.\(^5\) of substantial quantities of neutrophilic \(\alpha\)-naphthol acetate and butyrate NSE activity that is at least partially fluoride sensitive supports these findings. Why this activity is not usually demonstrable, or why it is largely restricted to the neutrophil precursors, is unknown.

We perform the NSE stains in "benign" monocytes, but the potential hematologic malignancies are more intensively studied. Therefore the fact that strong neutrophil NSE positivity has been recognized only in hematopoietic dysplasias or myeloproliferative disorders may be misleading. It seems clear, however, that the recognition of this phenomenon—demonstration of NSE in early-middle maturation forms of neutrophils—may be an ancillary aid in the early identification of this group of patients.

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REFERENCES

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Neutrophilic Nonspecific Esterase: Reply

To the Editor:

In our cytochemical evaluation of acute leukemia specimens, we routinely use the AS-D-chloroacetate esterase (AS-D-CE) and α-naphthyl esterase (NSE) tests utilizing the “combined” method of Yam et al. Although technical problems occur more frequently with the combined method than when each esterase test is done separately, there are nonetheless obvious advantages. When done separately, there are nonetheless obvious advantages to the former. First, individual cells can be shown to have both NSE (monocyte) and AS-D-CE (neutrophil) reactivity as we described.2 Second, there may be slightly increased efficiency in having two stains on one slide. Third, the “background” of NSE in myeloid cells is much fainter (very weak to absent), obviating the need for routine fluoride inhibition.1 2 Presumably Dr. Rydell tested separate esterase stains, which indicates that his observations may not be directly comparable to ours for technical reasons. Certainly we have noted more intense reactivity with NSE and AS-D-CE in individual stains; therefore terms such as “strongly” and “moderately” cannot be interpreted outside of the context of the technology employed.

An additional comment about the fluoride sensitivity of Dr. Rydell’s “strongly or moderately” NSE-positive neutrophil precursors seems warranted. Evaluations of specimens from normal persons (21) and chronic granulocytic leukemia1 patients suggest that whatever minimal NSE is present in myeloid cells is at most weakly inhibited by fluoride. On the other hand, the NSE reactivity in a typical monocytic2 or the myelomonocytic in acute myelomonocytic leukemia (3) is very sensitive to inhibition by fluoride. Dr. Rydell reports marked fluoride sensitivity of the NSE in the four specimens he examined. Furthermore, the nature of the “unclassified” myeloproliferative disorders is vague, leading us to wonder if some might be acute myelomonocytic leukemia in evolution.

The crux of the issue is undoubtedly whether or not one uses the standard morphology or the cytochemistry as the landmark for identifying the cells. Our own bias3,4 has been that the presence of a given enzyme makes a stronger statement about the function and physiology of the cell than does the morphology; since the response to cytotoxic agents is also related to the “physiology” of the cell, we believe that the cytochemical characteristics may be more relevant than the morphology to the prognosis of subtypes of nonlymphocytic leukemia. An analogy might be the vastly different function and response of normal and abnormal T and B lymphocytes, which are classified solely on the basis of functional markers because there is little if any morphologic difference between the two. The argument as to whether standard morphologic or cytochemical characterization is the most biologically relevant is inevitably circuitous, but the advent of more effective therapy for patients with acute leukemia should provide an atmosphere for a reexamination at the clinical level of the cytochemistry versus morphology controversy.

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REFERENCES

Neutrophilic nonspecific esterase [letter]

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