As noted by Dr. Fumarola, Limulus amebocyte lysate can be used to remove endotoxin from erythropoietin without loss of hormone activity. However, such preparations may not be suitable for tissue culture work due to the presence of residual amebocyte protein. Finally, even with removal of the endotoxin, the ratio of active hormone to contaminating protein in these crude preparations remains approximately 1:200 to 1:5000. Clearly, both removal of endotoxin and complete purification of erythropoietin will be required before definitive studies of its physiologic effects can be undertaken.

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REFERENCES

Counting of Monocytes

To the Editor:

Loos et al. described a technique for accurately counting monocytes based on size discrimination. Two methods were used: (1) the detection of light scatter signals by flow cytometry (Cytograf, Bio/Physics Systems, Westwood, Mass.) and (2) electrical resistance changes as measured by the Coulter Counter (Model ZF with pulse-height analyzer). These methods require the use of purified mononuclear leukocyte preparations since red cells, platelets, and granulocytes interfere with the procedure. When compared to manual counts, correlation coefficients of 0.97 for the Cytograf and 0.96 for the Coulter method were reported.

The authors emphasized the applicability of this method to detect the percentage of contaminating monocytes in Ficoll-Hypaque prepared mononuclear cell preparations. They confirmed the identity of monocytes by lysozyme content and phagocytic capability. They did not stain for nonspecific esterase, which is generally considered an excellent marker for cells of the monocyte series. The authors were also unaware that we had previously reported a Cytograf esterase technique for counting monocytes and for assaying for monocyte esterase activity which does not require separation of leukocytes from other formed elements but can be used directly on whole blood. They
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reported finding two populations of monocytes, a majority large-sized population and a minority small-sized population.

It is of interest that recent studies from our laboratory indicate that some hospitalized patients have a dual population of monocytes, one with high esterase activity and the other with low activity. Furthermore, monocytes with high activity have a higher scatter signal than monocytes with low activity. Since the scatter signal is generally related to size, our findings support those of Loos et al. The relationship of such subclasses of monocytes to function and to the immune response is an intriguing and yet unanswered problem. These approaches to the study of monocyte size, number, and enzyme content yield results which are unobtainable by manual counting methods and make it possible not only to determine the presence of monocytes in Ficoll Hypaque lymphocyte preparations but also permit accurate clinical interpretations of relatively small alterations in peripheral blood monocyte counts. Similar interpretations can be obtained from 10,000 cell monocyte counts derived by the automated differential cell counter, Hemalog D (Technicon, Tarrytown, N.Y.), which also utilizes a cytochemical esterase marker for monocytes.

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Counting of Monocytes: Reply

To the Editor:

I apologize for the fact that I could not incorporate the beautiful work of Dr. Kaplow in my article since his article appeared when my work had been submitted for publication.

I greatly appreciate that this latest work now confirms our data on the heterogeneity in size and density of peripheral blood monocytes and that this heterogeneity is also expressed in the esterase activity of these cells. Indeed, our method of monocyte counting requires the preparation of mononuclear cells. This requirement may be a disadvantage in clinical use. For immunologic research, however, the preparation of mononuclear leukocytes is required, and in particular in the latter type of work an accurate analysis of the composition of the cell preparation is of great importance. Monocyte counting based on sizing only has the advantage that the true Coulter volume or size is more accurately approached since no fixation or staining techniques are involved. We have shown that in particular the size of the lymphocytes and monocytes is of crucial importance for cell separations. Moreover, monocyte counting based on sizing can be performed with relatively cheap and simple equipment.

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Counting of monocytes [letter]

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