13. Tubbs RR, Valenzuela R, Savage RA,

The Authors’ Reply

To the Editor:—We read with interest the communication by Wheeler and associates regarding our paper describing surface markers in pure monocytic (M5) leukemia.4 The phrase “prominent spontaneous erythrophagocytosis by peripheral blood and marrow atypical promonocytes and monoblasts, as in Case 1” referred to the numbers of monoblasts engaged in phagocytic activity and not to the frequency of phagocytosis in the entity of acute monocytic leukemia, as Wheeler and associates seem to imply. The comments of Wheeler and associates regarding erythrophagocytosis by leukemic monoblasts seem directed at the frequency of occurrence of phagocytic activity in acute monocytic leukemia rather than the degree of phagocytosis observed in any individual case. However, neither the article cited8 nor several commonly available hematology textbooks2,3 support the contention of Dr. Wheeler and his associates that erythrophagocytosis is “uncommon but not rare in such cases.”

The relevant statements from two of the references quoted by Dr. Wheeler read as follows: “... rare leukemic monocytes demonstrating phagocytosis of erythrocytes... were identified during the later portion of the clinical course,” and “... rarely, marked phagocytosis is evident as indicated by the presence of engulfed red corpuscles in the cytoplasm.” For far more common than the massive erythrophagocytosis observed in our Case 1 is the finding of an occasional erythrophagocytic blast as shown in our Case 2.

We fully agree with the statement that erythrophagocytosis is a non-specific finding that may be observed in a variety of hematologic and even in nonhematologic malignancies. We discussed the nonspecificity of erythrophagocytosis in a communication4 not cited by Wheeler and associates in their literature review, which appeared in the issue of the American Journal of Clinical Pathology following the one containing the paper on acute monocytic leukemia.

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References

The Dextran Sulfate–MgSO₄
Precipitation as a Method for Quantitation of High-Density Lipoprotein Cholesterol

To the Editor:—Henderson and coworkers in a recently published article6 claimed that the dextran sulfate–MgSO₄ precipitation method results in artifically lower HDL cholesterol concentrations when the cholesterol determination is carried out at ambient temperature on automated analyzers using enzymatic cholesterol reagents. They speculate that the precipitating reagents affect either the enzymes present in the assay or the availability of the lipoprotein cholesterol to the enzymes.

In their study, Henderson and colleagues do not report that they have investigated the compatibility of the various precipitating reagents, enzymatic cholesterol reagents, and automated instruments that are used in conjunction to make the analytic de-
The difference between the means is 2.6%.

Henderson and colleagues do not specifically state all the parameters used to perform the cholesterol determinations. They do mention that 10-min incubations were allowed for cholesterol determinations at ambient temperature on the Gilford® 3500. This is not sufficient time for the reaction to proceed to completion, and low HDL cholesterol concentrations will result.

We have performed extensive comparative studies on the HDL cholesterol concentrations determined by the Dow® manual cholesterol method at 37 C and the method adapted to the Gilford System 3500 and the ABA-100 Bichromatic Analyzer®. The program card for the Dow HDL cholesterol determination on the Gilford 3500 requires a 20-min incubation at 25 C and a sample/reagent ratio of 1:41, whereas the ABA-100 requires a 10-min incubation at 37 C and a sample/reagent ratio of 1:26 for optimal results. A comparison of the HDL cholesterol concentrations, determined with the Dow enzymatic cholesterol reagent, using the manual method (X) and the methods adapted for the two analyzers (Y) has resulted in the following linear regression analysis of the data:

\[
ABA-100 \ Y = 1.03x - 0.24 \\
Mean \ Y = 46.2 \text{ mg/dl} \\
Mean \ X = 45.0 \text{ mg/dl} \quad r = 0.993 \\
N = 27 \\
Gilford 3500 \ Y = 0.998x - 0.15 \\
Mean \ Y = 45.1 \text{ mg/dl} \\
Mean \ X = 45.4 \text{ mg/dl} \quad r = 0.992 \\
N = 26
\]

The Dow HDL cholesterol method has been adapted to several other automated analyzers that perform the cholesterol determination at ambient temperature. The parameters used have been determined by extensive optimization and development studies for each analyzer. In each case, close agreement with the manual method has been obtained.

Further studies have been performed in which serum cholesterol concentrations were determined enzymatically in the presence of the precipitating reagents at the concentration that is present when the HDL cholesterol supernatant is assayed in the manual test (i.e., 1:31 dilution of the supernatant concentration of the precipitating reagents). These extremely low precipitating reagent concentrations did not seem to produce any visible turbidity in the reaction mixture, owing to precipitation of serum lipoproteins when the serum was introduced into the cholesterol reagent. In these studies, the effect of both the dextran sulfate–MgSO₄ reagents and the heparin–MnCl₂ precipitating reagents were examined for comparison. Dow enzymatic cholesterol reagent was reconstituted with the original reconstituting reagent, with reconstituting reagent containing 3 mmol/l MgSO₄ and 28 mg/l dextran sulfate, or reconstituting reagent containing 1.5 mmol/l MnCl₂, 5,900 units/l sodium heparin (derived from porcine intestinal mucosa) and 4 mm EDTA. A serum specimen with a total cholesterol concentration of 415 mg/dl was assayed at 25 C and 37 C in temperature-controlled spectrophotometers. The rate of the reaction was monitored, recording the absorbance at 1-min intervals. The results of the duplicate assays were averaged.

The data in Table 1 show that the rates of completion at a given temperature 25 C or 37 C do not differ significantly between the reagents, indicating that the presence of dextran sulfate and MgSO₄ does not have an inhibitory effect on the rate of the enzymatic reactions. The data also show that among the reagents virtually no difference exists between the absorbance read after 20-min incubation at 25 C and the absorbance read after 10-min incubation at 37 C, indicating that the presence of dextran sulfate and MgSO₄ does not

Table 1. Analysis of the Effect of Temperature on the Rate of the Cholesterol Assay

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Reagent without Precipitating Reagents</th>
<th>Reagent with Dextran Sulfate and MgSO₄</th>
<th>Reagent with Heparin and MnCl₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>25 C</td>
<td>% Completion at 10 min: 95.0</td>
<td>% Completion at 20 min: 99.0</td>
<td>% Completion at 10 min: 98.0</td>
</tr>
<tr>
<td></td>
<td>% Completion at 5 min: 93.6</td>
<td>% Completion at 10 min: 98.0</td>
<td>% Completion at 10 min: 98.0</td>
</tr>
<tr>
<td>37 C</td>
<td>% Difference between absorbance*</td>
<td>2.7</td>
<td>2.2</td>
</tr>
</tbody>
</table>

* Absorbance refers to the net absorbance obtained after subtraction of reagent blank.
reduce the availability of lipoprotein cholesterol to the enzymes when the reaction is performed at 25°C and allowed to proceed to completion.

It has been known for several years that enzymatic cholesterol reagents containing a phosphate buffer will react with Mn²⁺ ion in heparin-MnCl₂ supernatants to form a precipitate that will result in artifactually high and variable HDL cholesterol concentrations. Although Henderson and co-workers state in the section on reagents that EDTA was added to the Abbott® cholesterol reagent, they later state in their results that there was no addition of EDTA to the reagent. This may explain the high HDL cholesterol concentrations they obtained using the heparin-MnCl₂ method.

The authors have also reported an apparent decrease in HDL cholesterol concentration when dextran sulfate and MgSO₄ are added to HDL isolated by ultracentrifugation and dialyzed against 0.15 mol/l NaCl. Others²,²,⁷ have shown a similar result when heparin and MnCl₂ are added to isolated HDL. Burnstein and colleagues³,⁴ have discussed at length the probable reasons for this phenomenon, which include the effect of low ionic strength as well as the absence of serum proteins, which may facilitate the precipitation of HDL. This effect is common for polyanions that are sulfated polysaccharides,²,⁴ but is not pertinent since these precipitating reagents are applied to whole serum in most clinical applications—not to isolated lipoprotein fractions.

In summary, the dextran sulfate-MgSO₄ method can be used with enzymatic cholesterol reagents on automated analyzers at ambient temperature to quantitate HDL cholesterol accurately if the proper instrument parameters are used. The results of Henderson and co-workers are indeed artifacts, not of the precipitation methods, but of their choice of analytic methodology.

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References

The Authors' Reply

To the Editor:—Thompson and Roy justifiably underscore the necessity to document the compatibility of equipment and reagents in validating analytic determinations. In response to their letter, we recently assayed ten serum samples in quadruplicate using both heparin-Mn²⁺ and dextran sulfate-Mg²⁺ precipitation. Dow® Diagnostics provides a technical bulletin for HDL cholesterol determination (Product Code 45264) to obtain the dextran sulfate-Mg²⁺ HDL cholesterol supernatant, and the Dow Enzymatic Cholesterol Reagent (Product Code 46650) our mean cholesterol values were 16% lower than those obtained under identical conditions with the use of the heparin-Mn²⁺ precipitation method. Moreover, the Hyland HDL cholesterol standard serum (Omega® Lipid Fraction Control Serum Lot 4610Y001A) with a value of 30 mg/dl had an apparent value of 23 mg/dl after dextran sulfate-Mg²⁺ precipitation, 29 mg/dl after heparin-Mn²⁺ treatment.

The program card for the Gilford System 3500 cannot require a 20-min incubation at 25°C as indicated by Thompson and Roy. The instrument operates only at ambient temperature, even though the cuvette itself is thermostated at 25°C.

With respect to the apparently contradictory findings of Thompson and Roy we offer the following comments. (1) Preparative ultracentrifugation of HDL requires two consecutive steps. Recoveries of greater than 90% are most unusual even with extraordinary precautions. (2) We per-