placed into a laboratory oven for over-night drying at 50 °C. We currently use an average of 21 rotors per day, which take less than 30 min to wash with less than 10 min of operator time. We find that the rotors can be washed more than 50 times without any problems. We have tested the effectiveness of the washer by measuring absorbance detected after washing cuvettes filled with highly colored food dye and have found the wash process to be thorough.

The total cost of the rotor washer was less than $75. The annual cost savings achieved by washing rotors is enormous: based on our current use of 21 rotors per day it exceeds $16 000 per year.

There are no patents on the rotor washer and the reader is encouraged to use or market the unit. Detailed blueprints of the washer are available from the authors.

References

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Simple Removal of Lipids from Serum

To the Editor:

Lipemia in blood samples is a common problem in the clinical laboratory, and electrolyte management in the acutely ill patient is often complicated by analytical inaccuracies caused by interference by high concentrations of lipids in the serum. For example, hypoponatremia and hypokalemia are hard to gauge in a patient whose serum is milky because of diabetic ketoacidosis. Ultracentrifugation removes lipoproteins and "clears" lipemic samples for accurate determination of electrolytes (1), but this takes several hours and is obviously not appropriate when rapid management decisions are needed.

This experience with ultracentrifuged samples has led to helpful formulas (2) to correct for artificial electrolyte abnormalities due to lipemia, and such corrections are commonly used. We now report a simple, relatively rapid technique for removing lipids from the serum, which permits more accurate determination of non-protein-bound analytes. We found that use of polyethylene glycol (PEG), an inexpensive agent commonly used to precipitate immunoglobulins (3), is effective in removing lipoproteins.

To the serum sample, 1.5 mL or less, in a glass tube, add an equal volume of PEG, 250 mL/L. Vortex-mix thoroughly, then separate the two phases by centrifugation (2300 rpm, 20 min). The lipids pass into the PEG (lower) phase, which is now milky white and separated by a sharp interface from the n-clear serum sample. Use this clear supernate for analyte determinations, but multiply the results by two to correct for the dilution.

We used this technique with 15 lipemic samples from patients. The mean cholesterol concentration was 4.06 (SE 0.47) g/L before treatment with PEG and 200 (SE 20) mg/L after. The concentration of triglycerides was decreased to 250 (SE 60) mg/L from 15.07 (SE 2.48) g/L. By comparison, ultracentrifugation for 10 h decreased these values to 1.68 (SE 0.24) g/L and 3.7 (SE 0.62) g/L, respectively.

Table 1 compares values for glucose, urea nitrogen, sodium, potassium, chloride, and bicarbonate in untreated samples, PEG-treated samples, and ultracentrifuged samples. Removal of lipids by PEG markedly increased the values of all analytes. The ultracentrifuged samples tended to show smaller increases, because of the less-complete lipid removal.

We caution that the PEG technique is not appropriate when enzyme activities or protein-bound analytes such as calcium or thyroxin are to be measured in serum.

References

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Short-Term Changes in Iron, Ferritin, Total Iron-Binding Capacity, and Transferrin in Serum after Myocardial Infarction

To the Editor:

As known for many years, hypoferremia follows myocardial infarction (1). More recently, it was suggested (2) that, when used in association with enzyme activity assays, results for serum iron might prove useful in diagnosing myocardial infarction, and that its rapid decrease between 24 and 48 h after admission was in some way associated with stress. However, the standard biochemical stress of an insulin hypoglycemia test did not result in hypoferremia (3). This apparent conflict might merely reflect the time scales of the two studies. We wished to resolve this point and, more importantly, to assess the changes during the first few hours after myocardial infarction because, ideally, any diagnostic test should be applicable as soon as possible after clinical presentation.

We measured iron, ferritin, total iron-binding capacity (TIBC), and transferrin in serum of patients with myocardial infarction during the 48 h after admission. The subjects were 21 patients who were admitted to the Coronary Care Unit and who had given informed consent to have multiple blood specimens collected as part of a trial for a new calcium antagonist drug. There were 19 men, ages 36 to 67 years (mean, 57 years), and two women, ages 62 and 68 years. All were admitted to hospital within 3.5 h of

Table 1. Mean and SE for Analytes in Lipemic, PEG-Treated, and Ultracentrifuged Samples

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Lipemic SE</th>
<th>PEG-treated SE</th>
<th>Ultracentrifuged SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose, g/L</td>
<td>2.02 ± 0.28</td>
<td>2.22 ± 0.29*</td>
<td>2.12 ± 0.29*</td>
</tr>
<tr>
<td>Urea, N, g/L</td>
<td>0.122 ± 0.024</td>
<td>0.152 ± 0.023*</td>
<td>0.126 ± 0.026</td>
</tr>
<tr>
<td>Sodium, mmol/L</td>
<td>136.9 ± 1.8</td>
<td>144.9 ± 2.0</td>
<td>140.7 ± 1.8</td>
</tr>
<tr>
<td>Potassium, mmol/L</td>
<td>4.2 ± 0.3</td>
<td>4.7 ± 0.2</td>
<td>4.6 ± 0.3</td>
</tr>
<tr>
<td>Chloride, mmol/L</td>
<td>97.8 ± 1.5</td>
<td>104.9 ± 1.6*</td>
<td>101.0 ± 1.8</td>
</tr>
<tr>
<td>Bicarbonate, mmol/L</td>
<td>19.2 ± 1.4</td>
<td>21.2 ± 0.8*</td>
<td>19.3 ± 1.3</td>
</tr>
</tbody>
</table>

* Significantly different from lipemic (untreated) samples at p < 0.05 and p < 0.01.
onset of chest pain. None had heart failure, severe renal or hepatic dysfunction, aortic stenosis, hypotension, or increased heart rate, and none was taking cardioactive drugs.

Venous blood was sampled every 4 h for the first 24 h of admission, and then every 8 h for the next 24 h. Each specimen was centrifuged as soon as the clot formed, and the serum was separated and stored at −20 °C until analysis.

We measured iron in a Rotochem IIA parallel centrifugal analyzer (V.A. Howe Co., Ltd., London, U.K.), using Ferrozine [3-(2-pyridyl)-5,6-bis(4-phenylsulfonic acid)-1,2,4-triazine] in an acetate buffer containing thioglycolic acid. Serum ferritin was measured with reagent kit sets (Amersham International plc, Amersham, U.K.).

TIBC was measured by saturation of the binding sites with ferric chloride, removal of excess iron with magnesium carbonate, and assay of iron as described above. Transferrin was measured immunometrically in the Rotochem IIA analyzer with use of antibody from SAPU, Law Hospital, Carluke, Scotland.

The imprecisions (SD) of the iron, ferritin, TIBC, and transferrin measurements, judged from duplicate analysis of patients’ specimens, were: 1.51 μmol/L (n = 110), 2.1 μg/L (n = 143), 2.97 μmol/L (n = 73), and 0.04 g/L (n = 157), respectively.

We assayed the activity of the myocardial isoenzyme (CK-MB) of creatine kinase (EC 2.7.3.2) in a DuPont acx III discrete analyzer. All subjects had an increase in CK-MB to more than 100 U/L, showing that all had suffered a myocardial infarction (normal reference range: up to 10 U/L).

Table 1 summarizes our analyses. Although hyperferremia does develop after myocardial infarction, it is insignificant until 24 to 32 h after onset of symptoms. Interestingly, iron concentrations increased slightly during the first 12 h, although not significantly. This phenomenon has been seen after surgery (4), but was attributed to autotransfusion of blood and the resulting traumatization of erythrocytes. Perhaps mild hyperferremia does in fact occur before hyperferremia in all the stressful events that result in low iron concentrations (5), although there is some evidence that iron concentrations fall after surgery within 6 h (6).

Ferritin concentration increased significantly after 40 to 48 h, as it does after surgical stress (4, 7, 8). TIBC values did not change significantly during the 48 h after infarction but, in contrast to the short-term changes after surgery (6), transferrin concentrations declined significantly only at 32 to 40 h.

We conclude that specimens for investigation of iron status in relation to myocardial infarction must be taken within 12 h of the onset of chest pain. Moreover, the decline in serum iron, which has been termed “rapid” (2), is unlikely to occur early enough to be a useful adjunct to existing tests. Our present findings support our earlier data (3) that values for serum iron and TIBC remain essentially unchanged shortly after stress. Our finding that a significant decrease in serum iron occurs before a significant increase in ferritin does not support suggestions (4, 9) that increased ferritin synthesis is the primary event that leads to retention of labile iron and a decrease in the early phase of iron release.

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References


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25-Hydroxyvitamin D_2_ and 25-Hydroxyvitamin D_3_ as Measured by Liquid Chromatography and by Competitive Protein Binding

To the Editor:

Measurement of 25-hydroxyvitamin D_2_ [25(OH)D_2_] and 25-hydroxyvitamin D_3_ [25(OH)D_3_] has been reported by several laboratories that measured absorbance of the effluent from the normal-phase "high-performance" liquid chromatography (1, 2). We have had a problem quantifying 25(OH)D_2_ because a large absorption peak appears between the two peaks that represent 25(OH)D_2_ and 25(OH)D_3_. This peak appeared frequently in chromatograms of control samples that had been stored at −60 °C and assayed within two weeks. We undertook to determine the extent to which this peak interfered with measurement of 25(OH)D_2_ and 25(OH)D_3_ by absorbance during liquid chromatography as compared with competitive protein binding (CPB) assay, and to develop reliable techniques for assaying these metabolites in plasma containing the interfering peaks.

We isolated column effluent corresponding to the 25(OH)D region from plasma, using a modification of the method described by Lambert et al. (3). We extracted aliquots of plasma with methanol:methylene chloride (2:1 by vol) and resolved the 25(OH)D-containing fractions by chromatographing these plasma extracts on 0.8 × 15 cm columns of Sephadex LH-20 (Pharmacia Inc., Piscataway, NJ), using the solvent system of hexane:chloroform:methanol (9:1:1 by vol). Some fractions were further resolved by passage through a second such LH-20 column, with use of the equilibrium solvent

Table 1. Concentrations of Iron, Ferritin, TIBC, and Transferrin in 21 Patients at Various Times after Myocardial Infarction

<table>
<thead>
<tr>
<th>Time, h</th>
<th>Iron, μmol/L</th>
<th>Ferritin, μg/L</th>
<th>TIBC, μmol/L</th>
<th>Transferrin, g/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–4</td>
<td>13.2</td>
<td>1.4</td>
<td>195</td>
<td>38</td>
</tr>
<tr>
<td>4–8</td>
<td>13.5</td>
<td>2.7</td>
<td>160</td>
<td>30</td>
</tr>
<tr>
<td>8–12</td>
<td>13.7</td>
<td>3.1</td>
<td>149</td>
<td>31</td>
</tr>
<tr>
<td>12–16</td>
<td>14.1</td>
<td>3.2</td>
<td>219</td>
<td>37</td>
</tr>
<tr>
<td>16–20</td>
<td>12.1</td>
<td>2.3</td>
<td>248</td>
<td>60</td>
</tr>
<tr>
<td>20–24</td>
<td>10.8</td>
<td>1.3</td>
<td>236</td>
<td>48</td>
</tr>
<tr>
<td>24–32</td>
<td>9.1 *</td>
<td>0.8</td>
<td>242</td>
<td>51</td>
</tr>
<tr>
<td>32–40</td>
<td>8.1 *</td>
<td>1.7</td>
<td>316</td>
<td>84</td>
</tr>
<tr>
<td>40–48</td>
<td>6.2 *</td>
<td>0.8</td>
<td>404</td>
<td>77</td>
</tr>
</tbody>
</table>

*0.05 > p > 0.01. *0.01 > p > 0.001. * p > 0.001.