Determination of Calcium

The Editor:

Recently, in a Letter to the Editor, Fuchs and McIntosh (1) suggested that a side-by-side comparative investigation of ionized calcium as determined with the Orion SS-20 and Auto AMT Electrophoresis would be the only valid procedure for resolving the discussions concerning aerobic and anaerobic sample handling when ionized calcium (Ca²⁺) is being measured in serum (1, 2). Here, we report such a comparison.

Blood was collected from normal staff members by venipuncture without anesthesia into a plain evacuated blood-collection tube and centrifuged at 2000 rpm. After 30 min, two samples of serum were removed with 1-mL tuberculin syringes. All air was expelled from the syringes, and the syringes were capped. The pH of one sample was measured with a pH/Blood Gas Analyzer IL 813 (instrumentation Laboratory, Lexington, Mass. 02173). Ionized calcium was measured in the other anaerobic sample with the Orion SS-20. The remaining sample was not treated anaerobically, and was used to determine the ionized calcium with the AMT-Auto-Electron system (Applied Medical Technology, Menlo Park, Calif. 94025). The tube was filled as completely as possible, leaving no more than 0.5 ml of air (vacuum) over the blood. This resulted in only insignificant loss of CO₂ from the sample.

As discussed by Schwartz (2) the AMT-Auto-Electron system equilibrates aerobically handled serum with 5.22% CO₂ and measures pH and ionized calcium. It then calculates ionized Ca²⁺ to a given pH (set by the operator) according to an algorithm developed by Schwartz. Our values presented here are based on the pH measured with the IL Model 813, which is the pH under which the measurement in the Orion SS-20 was performed. The Orion SS-20 is equipped with one calcium ion-selective electrode, whereas the AMT Electrophoresis has two electrodes and values are printed out individually for both. The data therefore allowed a comparison between two AMT electrodes as well as the comparison between the Orion and the AMT electrodes. In 16 normal subjects, ages 26–66 years, nine men and seven women, with pH ranging from 7.31 to 7.45, we found with the SS-20 electrode 4.13 ± 1.2 (1 SD) mg of ionized calcium per liter (note: the measurements were made in duplicate). The values for the 2 Electrodes were 42.3 ± 1.1 and 42.1 ± 1.2 mg/liter, respectively. There is no significant difference among any of these three groups.

Another question raised by Fuchs and McIntosh (1) concerns the validity of the serum used in the AMT Electrophoresis system. We considered that, for comparative purposes, the best answer would be obtained by measuring the AMT standards with the Orion SS-20 as if they were regular specimens, after equilibration with CO₂ to pH 7.4. Four vials of AMT standards were tested this way with repeated measurements. For the 42 mg/liter standard we found 42.0 ± 0.7 (1 SD) mg/liter. We conclude that the Orion SS-20 and the AMT Electrophoresis give comparable results and identical precision. The choice of instrument thus depends on considerations such as the need for automation, and the usual sample volume (0.5 ml non-retrievable for Orion SS-20, 1.3 ml retrievable for the AMT).

Identification of any commercial product does not imply recommendation or endorsement by the Veterans Administration, nor does it imply that the material or equipment identified is necessarily the best available.

References


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Comparison of Agarose and Polyacrylamide Techniques for Lipoprotein Electrophoresis

To the Editor:

Quantitation of plasma lipoprotein fractions by electrophoresis is known to correlate with the quantitation of fractions prepared by ultracentrifugation. The relation was demonstrated by Hatch et al., using agarose gel as a support medium for the electrophoresis (1). In a more recent study, polyacrylamide gel was used (2).

We have compared the electrophoretic patterns obtained by using agarose and polyacrylamide gels. Agarose scans and the quantitative densitometry were carried out at the Donner Laboratory by the method of Hatch et al. (1). The qualitative polyacrylamide gel patterns were determined by use of the Ames Co. "Redi-Disc" polyacrylamide gel electrophoresis kit (3).

A comparison of some Type 2b patterns is given in Figures 1 and 2. In Figure 1 it is seen that patterns obtained with the polyacrylamide gel kit in cases of Type 2b hyperlipidemia (right-hand photographs) actually resemble the patterns expected for Type 4 (left, bottom photograph) and are not similar to the Type 2 patterns (left, middle). The examples are clearly of Type 2b, for the serum cholesterol concentrations and the quantitative values for beta-lipoproteins in serum are significantly increased (Table 1). Nevertheless, in the polyacrylamide gel patterns the beta

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1. We are indebted to Ronald M. Krause, M.D., and the staff of Donner Laboratory for carrying out the agarose gel electrophoresis and the corresponding quantitation of the lipoprotein fractions.
electrophoretic bands are fainter than in the normal.

There is no doubt that the polyacrylamide technique is more rapid than the agarose technique (4). However, we have found numerous cases in which the beta band does not appear with its expected characteristic intensity in keeping with the serum total cholesterol concentration. As stated in the manufacturer’s labeling, “Some Type 2b patterns with a strong pre-beta band resemble those of Type 4 so that other criteria may be required to establish a definitive phenotype.” In our experience the additional information is usually necessary. To obtain an accurate classification of phenotype, we have found that we must rely on the agarose gel electrophoresis method, which readily distinguishes 2b from 4.

We have not determined whether the illustrated decrease in the beta component on using polyacrylamide gel is the result of defective staining, lipoprotein migration, or other factors. However, it is known that the amount of stain in the polyacrylamide gel electrophoresis kit is low by comparison to the amount of stained moiety (that is, the amount of cholesterol) (2). Increasing the concentration of the stain provided in the kit may be necessary to obtain an accurate 2b pattern.

In comparing the Ames polyacrylamide kit with the Donner agarose

Fig. 1. Left: Reference polyacrylamide electrophoresis patterns, showing typical normal (top), Type 2a (middle), and Type 4 (bottom).
Right: Three polyacrylamide patterns compared to densitometer scans of the agarose patterns.

The integrated densitometer results are given in Table 1. Total cholesterol and beta-lipoprotein concentrations (Table 1) characterize the patients as 2b, although the polyacrylamide patterns resemble Type 4.
Table 1. Lipid Values for Specimens Illustrated in Figure 1

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Cholesterol</th>
<th>Triglyceride</th>
<th>Beta-lipoprotein</th>
<th>Prebeta-lipoprotein</th>
<th>Alpha-lipoprotein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Left</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>top (normal)</td>
<td>2.18</td>
<td>0.66</td>
<td>ND*</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>middle (2a)</td>
<td>3.73</td>
<td>0.76</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>bottom (4)</td>
<td>2.60</td>
<td>2.96</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Right</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>top (2b)</td>
<td>4.34</td>
<td>6.25</td>
<td>11.13</td>
<td>6.42</td>
<td>0.29</td>
</tr>
<tr>
<td>middle (2b)</td>
<td>3.86</td>
<td>10.00</td>
<td>6.87</td>
<td>10.58</td>
<td>0.77</td>
</tr>
<tr>
<td>bottom (2b)</td>
<td>3.79</td>
<td>7.90</td>
<td>9.01</td>
<td>9.46</td>
<td>2.83</td>
</tr>
</tbody>
</table>

Normal (recommended) limits
<2.70  <1.80  <0.60  2.50  >1.80
* ND, not determined.

Furthermore, two of the three specimens illustrated are low in alpha-lipoproteins. The agarose patterns show this. However, the polyacrylamide patterns, especially the middle one in the figures, appear to have normal levels. Further studies are needed in order to characterize the differences between the two techniques in their sensitivity to chylomicrons and the alpha-lipoproteins at low concentrations.

References
3. Ames Co., Division of Miles Laboratories, Inc., Elkhart, Ind. 46514.

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A Fast-Migrating Alkaline Phosphatase Band

To the Editor:
I read with interest the report by Hardin et al. (1), in which they concluded that the alkaline phosphatase-like activity in the albumin region on cellulose acetate electrophorograms is merely an artifact.

In our laboratory, we seldom separate alkaline phosphatase (EC 3.1.3.1) when the patient has abnormally high bilirubin concentration (>10 mg/liter). To confirm the observation by Hardin et al. we did an alkaline phosphatase isoenzyme study on three patients with serum bilirubin concentrations ranging from 50 to 120 mg/liter. Indeed, a fast-migrating band was found in all these patients. Our electrophoresis was also done on cellulose acetate strips and with an electrophoresis system all supplied by Helena Laboratories, Beaumont, Texas 77704.

One of their 27 patients who had a fast-migrating alkaline phosphatase band had a serum bilirubin concentration of less than 10 mg/liter. In the past eight months, I also observed the fast-migrating band in two patients who had normal serum bilirubin concentrations. One was a 37-year-old woman with granulomatous hepatitis proven by liver biopsy; the other was a 76-year-old woman with liver metastasis from pancreatic carcinoma documented by liver scan.

Although experimentally Hardin et al. could demonstrate that the bilirubin/albumin complex migrated in the albumin region, we cannot attribute all the fast-migrating bands to an artifact caused by this complex, because some patients with normal serum bilirubin values also have the fast-migrating band. The significance of this band requires further investigation.

Reference

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Lecithin:Cholesterol Acyltransferase Response to Dietary Cholesterol Loading

To the Editor:
There is continuing interest and controversy regarding the significance of the amounts of cholesterol consumed by man. Mistry et al. (1) reported a variable response (~5 to 20%) in 10 men fed cholesterol supplements of 750 mg/day. Quinto et al. (2) showed the metabolic response to increased amounts of absorbed cholesterol in man to be a decrease in cholesterol synthesis and an increase in its re-excretion. A marked variability was noted among