as the early eluting HbA₁ fraction. Citrate agar electrophoresis has been compared with cation-exchange chromatography (4). Therefore, we attempted to confirm the aforementioned data (1, 2) by subjecting affinity-chromatographed glycated hemoglobin eluates to electrophoresis on citrate agar.

A 20-year-old diabetic man had a value for HbA₁ by cation-exchange of 14.1% (normal range 5.5–8.5%). Hemoglobin electrophoresis on cellulose acetate (Helena Labs., Beaumont, TX 77704) was unremarkable. Hemoglobin F by alkali denaturation (5) was <1%. Hemolysates of the man’s erythrocytes were subjected to cation-exchange chromatography (Pasteur; Isolab Inc., Akron, OH 44321) and affinity chromatography (Glyc-Affin; Isolab). Eluates were concentrated (Amicon B-15 concentrator; Amicon Corp., Lexington, MA 02173) (6) and subjected to citrate agar electrophoresis (Helena Labs.). As Figure 1 shows, the glycated hemoglobin fraction was composed of hemoglobin with both HbA₁ and HbF mobility, whereas the HbA₁ hemoglobin fraction consisted of hemoglobin with only HbF mobility. We believe these results are consistent with those of Abraham et al. and Klenk et al. by showing that a substantial amount of affinity-chromatographed glycated hemoglobin has HbA₁ mobility on citrate agar electrophoresis corresponding to HbA₁ elution by cation-exchange chromatography. Therefore, the cation-exchange-chromatographed HbA₁ is not identical to the affinity-chromatographed glycated hemoglobin because the procedures measure a different variable. We conclude that affinity chromatography is superior to cation-exchange chromatography in this application because it more directly measures glycated hemoglobin.

References

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Serum Adenosine Deaminase Activity is Increased in Sarcoidosis

To the Editor:

Macrophages and T-lymphocytes, which accumulate in sarcoid granuloma (1), are rich in adenosine deaminase (ADA; EC 3.5.4.4) (2, 3). Thus it is of interest to determine whether serum ADA activity will reflect granuloma mass as it is believed does the serum activity of angiotensin 1-converting enzyme (ACE; EC 3.4.15.1), which is present in macrophages and epithelioid cells (1).

Accordingly, I measured the activities of ADA and ACE in serum of patients with biopsy-proven sarcoidosis: 18 untreated and 24 already receiving treatment with corticosteroids.

Serum ADA activity was measured in nonhemolyzed samples by a modification of the method of Giusti (4). I assayed serum ACE activity by the method of Cushman and Cheung (5), as modified by Lieberman (6) and by Taylor and Freeman (7), using a 30-min incubation.

There were no age- or sex-related differences in serum ADA activity in the 63 laboratory staff members I used as the reference group. However, the mean activity of serum ACE in the female subjects of a similar group was significantly lower than that for men by the two-tailed t-test (p = 0.005). The ratio of women to men in all the groups studied was approximately 6:4.

Table 1 shows that for each enzyme, the mean activity was highest in the active-disease group; in the treated group it was intermediate between the active group and the reference population. Because it had been reported that ADA or ACE activities in serum may increase in some cases of liver disease (8, 9), I examined the measured activities of alanine aminotransferase, alkaline phosphatase, and γ-glutamyltransferase in the serum from the active-disease group. Four of the 18 patients with untreated sarcoidosis showed above-normal activity of at least one enzyme without any clinical features of liver disease, but neither the ADA nor the ACE activities were significantly different from those in the remaining 14 patients.

Seventeen of the 18 patients showed an abnormal serum activity of either ACE or ADA or of both. Only 13 patients showed an abnormal serum ACE activity. The serum activities of six patients were disparate: in four ADA was abnormal and ACE normal, and in two ACE was abnormal and ADA normal. (ACE activities were designated normal according to the reference range of the appropriate sex.) This demonstrates the advantage of measuring both ADA and ACE activities in serum from suspected cases of sarcoidosis.

These observations suggest that serum ADA assay is useful for detecting sarcoidosis, bearing in mind that increased activities are not specific for this disease, nor even for granulomatous diseases, being seen also in patients with liver disease, some lymphomas, and autoimmune diseases (10–12).

Table 1. Serum Enzyme Activities (U/L)

<table>
<thead>
<tr>
<th></th>
<th>ADA</th>
<th>ACE</th>
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<tbody>
<tr>
<td>Mean</td>
<td>SD</td>
<td>n</td>
</tr>
<tr>
<td>Healthy people</td>
<td>14.2</td>
<td>4.2</td>
</tr>
<tr>
<td>Untreated patients</td>
<td>21.2</td>
<td>29.2</td>
</tr>
<tr>
<td>Treated patients</td>
<td>12.1</td>
<td>7.1</td>
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</table>

Fig. 1. Results of citrate agar electrophoresis of hemoglobin

The anode and origin are at the right. At the top (1) is an HbFASC control. Beneath (2) is the patient's hemolysate. 3 and 7 (arrows) are the glycated hemoglobin eluates. 4 and 6 (arrows) are the HbA₁ eluate, 5 is the nonglycated hemoglobin eluate, and 8 is the other, later-eluting, hemoglobins in the HbA₁ assay. Note the two bands with HbF and HbA₁ mobility in 3 and 7.
I am grateful to Dr. G. Walker for helpful comments and to the staff of the Clinical Chemistry departments of the University and City Hospitals in Nottingham for providing facilities.

References


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Seven Ferritin Kits Compared with Respect to the "Hook" Effect

To the Editor:

Since the early 1970's, immunoradiometric assays for determination of ferritin in serum have been described, and various commercial kits have been manufactured by several companies. Immunoenzymometric assays have also become available recently.

Such assays are subject to a high-dose "hook" effect, which can result in underestimation of high ferritin concentrations and, indeed, several such cases have been reported.

We evaluated the performance of seven kits for determination of serum ferritin with respect to the high-dose hook effect:

<table>
<thead>
<tr>
<th>Ferritin concn, mg/L</th>
<th>Kit</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
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We supplemented a serum sample with purified liver ferritin to give a concentration of 60 mg/L. It then was diluted in human serum with a low ferritin content and these dilutions were assayed. All assays were performed according to each manufacturer's protocol. Table 1 summarizes the results.

Kits A and B do not show a high-dose hook effect up to 60 mg/L; all the others show it at some concentration, some demonstrating such a pronounced "hook" that a sample containing 60 mg/L would be interpreted as containing only 0.1 mg/L. Clearly, avoiding the high-dose hook effect is of the utmost importance when evaluating patients with non-iron-deficiency anemias, who may actually present with an iron overload, as well as those with iron-storage disease. Reporting a serum ferritin value in the normal range for these patients when the true value may be extremely supranormal would obscure the clinical picture and make more difficult a definitive diagnosis.

We conclude that all seven kits evaluated have obviously been developed with simplicity of operation as an important goal, which has been achieved to a considerable degree. However, Kits A and B seem to be superior to the rest with respect to the hook effect. The possibility of misdiagnosis, even of a relatively small proportion of patients, should make this an important criterion in the selection of a ferritin kit for use in routine testing.

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Table 1. Radioactivity (Counts per Minute) and Absorbance Readings (A) for Ferritin Concentrations of 0.05 through 60 mg/L

Determination of Free Thyroxin and Triiodothyronine in "Subclinical" Hypothyroidism and Hyperthyroidism

To the Editor:

The amounts of circulating non-protein-bound thyroxin (FT4) and triiodothyronine (FT3) presumably reflect biological activity more closely than do the total amounts of the respective hormones (TT4, TT3). Recently developed "single-step" radioimmunoassays involving the use of labeled analog hormones avoid time-consuming dialysis procedures and have therefore enhanced interest in determination of free thyroid hormone concentrations as a routine screening test. We were interested to see whether the determination of FT4 and FT3 could replace the thyroliberin (TRH) test (400 μg, administered intravenously) in establishing the diagnosis in patients with "subclinical" hyperthyroidism or hypothyroidism, i.e., patients with normal concentrations of TT4 and TT3 but sup-