ing the digitonide: first dissolve the washed precipitate of cholesterol digitonide in 0.4 ml of acetic acid in capped tubes in a 50 °C bath and then dilute to 2.0 ml with isopropanol.

This modification has been used regularly in our research laboratory and exhibits the excellent linearity expected of any standard method. Analysis of 27 standard curves of four values each, run during a year, yielded a regression line of slope = 0.996, ordinate intercept of \(-0.446 \text{ mg/dl}\), and a correlation coefficient \(r = 0.999\). Control samples (n = 25) taken the entire procedure were reproduced with a CV of 2.5% over the same period of time.

I hope that this information will be of help to those wishing to run a semi-automated determination of cholesterol digitonide, whether for purposes of comparing values so obtained with newer methodologies, or for the intrinsic value of the classical method itself.

References

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Sex-Related Differences in the Reference Values for Erythrocytic δ-Aminolevulinic Acid Dehydratase Activity

To the Editor:

The erythrocytic enzyme δ-aminolevulinic acid dehydratase (ALAD; porphobilinogen synthase, EC 4.2.1.24) is remarkably sensitive to lead exposure (1, 2). It has therefore been proposed for at least a decade that ALAD activity be used as an index of lead intoxication (3, 4) whether acute or chronic (5) or subclinical (6).

ALAD activity is inherited in a co-dominant fashion, resulting in a broad range of normal enzymatic activity (7). Using our technique we observed a two-fold range of normal ALAD activity. In addition, we found a significant difference between normal men and women in mean ALAD activity.

Blood was obtained from normal, healthy, nonmedicated men and women volunteers, 18 to 70 years old, by venipuncture into sodium heparinized Vacutainer Tubes. As a routine screen of the hematologic indices of the volunteers, complete blood counts were done with the Coulter Counter Model S (Coulter Electronics, Hialeah, Fla.) and microhematocrits (Hct) and reticulocyte percentages were determined (8).

Erythrocytic ALAD activity was determined by a modification of previously described micromethods for whole blood (9, 10). A 0.1-ml aliquot of heparinized whole blood was hemolyzed in 1.5 ml of a 2 ml/liter solution of the surfactant Triton X-100. The hemolyzate was warmed to 37 °C for 5 min and 1.0 ml of freshly prepared 0.01 mol/liter ALA-HCl in 0.25 mol/liter dibasic sodium phosphate/citric acid buffer at pH 6.7, also warmed to 37 °C, was added. After 1 h of incubation at 37 °C the reaction was stopped by adding 1.0 ml of trichloroacetic acid (100 g/liter) containing 0.02 mol of N-ethylmaleimide per liter, followed by 0.05 ml of a saturated copper sulfate solution. After standing for 5 min the mixture was centrifuged at 600 \( \times \) g for 10 min. A 2.0-ml aliquot of modified fresh Ehrlich's reagent (11) was added and the absorbance at 555 nm was measured after 15 min, with use of a cuvet with 1-cm light path in a colorimeter set to 100% transmittance with a reagent blank in which water was substituted for blood. A milliunit (mU) of ALAD activity has been defined as that activity resulting in the transformation of 1 nmol of δ-aminolevulinic acid (ALA) to porphobilinogen (PBG) per minute at pH 6.7 and 37 °C. Erythrocytic ALAD activity is expressed as milliunits per milliliter of packed erythrocytes and is determined by the following formula:

\[
\text{ALAD, mU/ml} = \frac{A_{60}}{(100/\text{Hct}) \times 40.56}
\]

where \(A_{60}\) = absorbance after 1 h at 37 °C and Hct is the hematocrit. The factor 40.56 is derived as follows.

\[
40.56 = \frac{(3.65 \times 2) (\text{blood dilution})}{0.1(\text{blood volume}) \times 60(\text{time}) \times 1 \times 0.060(\text{A of 1 nmol PBG/ml})} \times 2
\]

The significance of a difference between sample means was evaluated by Student's t-test.

Table 1 gives the reference values obtained for the sample population studied. There was no statistical difference between the mean male and female ages and reticulocyte percentages, but there was the expected highly significant difference in mean hemoglobin and hematocrit values with regard to sex, values for men being about 10% higher than those for women. In contrast, the mean erythrocytic ALAD activity in women was found to be significantly (about 10%) greater than that of the men.

The biological significance of the present finding is uncertain at this time, since it has been suggested that there may be excess enzyme in the body (12). However, this sex-related difference may reflect higher concentrations of available cofactors such as zinc, which has been demonstrated to stimulate erythrocytic ALAD (13–15). In any event, this sex-related difference should be considered when establishing reference values for the erythrocytic ALAD assay.

References
8. Miale, J. B., Laboratory Medicine: Re-

Table 1. Some Reference Values (Mean ± 2 SD) for Normal Human Erythrocytic ALAD Activity

<table>
<thead>
<tr>
<th>No. subjects</th>
<th>Age (a) ± range</th>
<th>Hemoglobin (g/liter)</th>
<th>Hematocrit (%)</th>
<th>Reticulocytes (%)</th>
<th>Erythrocytic ALAD acyl. (mU/ml RBC)</th>
</tr>
</thead>
<tbody>
<tr>
<td>δ</td>
<td>57</td>
<td>36.3 ± 23.4</td>
<td>15.4 ± 5.6</td>
<td>45.5 ± 1.2</td>
<td>6.2 ± 1.2</td>
</tr>
<tr>
<td>ϕ</td>
<td>49</td>
<td>37.9 ± 29.6</td>
<td>13.6 ± 1.9</td>
<td>40.9 ± 6.0</td>
<td>1.2 ± 1.2</td>
</tr>
</tbody>
</table>

Significance level (P) < 0.001 not signific. < 0.001 not signific. < 0.01

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Evaluation of a New Radioimmunoassay for Carcinoembryonic Antigen

To the Editor:
A new radioimmunoassay system produced by Dainabot Radioisotope Laboratory in Japan was recently made available to us by Abbott Laboratories in Montreal. We compared results by this assay with those by the currently used CEA-Roche assay (Roche Diagnostics). CEA is not a cancer-specific antigen and is generally regarded as a cancer-related antigen (1); it is also generally recognized that its measurement in plasma may be used to monitor cancer patients during therapy (2).

The CEA-RIA kit offered by Abbott Laboratories is a solid-phase radioimmunoassay based on the work reported by Hirai and Nishi (3, 4). After a 1:1 dilution, 0.5 mL of the patient’s serum is heated at 85 ± 5 °C for 10 min; the antigen is present in the supernatant fluid obtained after centrifugation at 2000 × g, a 100-μL portion of which is added in duplicate to wells of a microassay plate. A filter-paper disk coated with goat anti-CEA is placed into each well. The same procedure is repeated with predetermined standards and a control sample included in the kit. The plate is agitated at room temperature for 4 to 6 h and the fluid is aspirated. After the paper disk is washed with physiological saline, 100 μL of 125I-labeled anti-CEA is added to all wells and the plate is agitated for at least 16 h at room temperature. After being washed with saline, the disks are transferred to properly labeled test tubes and the radioactivity is counted.

Using this system, we obtained an intra-assay standard deviation of 0.36 μg/Liter, a mean serum CEA value of 3.85 μg/Liter (n = 10), and a CV of 9.3%. Inter-assay determinations performed with the control sample included in the kit (CEA, 4 to 6 μg/Liter) gave a mean of 4.45 μg/Liter, with a deviation of 0.38 μg/Liter, which corresponds to a coefficient of variation of n = 6.

Because clinicians were already used to CEA values obtained by the Roche CEA assay, we measured CEA in sera of 94 patients by both assays. Because the Roche assay gives a plateau for values exceeding 20 μg/Liter (the evaluated assay gave a near-linear relationship for values varying from 0 to 100 μg/Liter), patients’ sera with values greater than 20 μg/Liter were excluded. The means were 4.24 (Roche) and 5.97 (Abbott) μg/Liter. The correlation coefficient was 0.70 and P < 0.001. When 17 sera were measured with the CEA-Abbott method in two different laboratories, a correlation of 0.98 (P < 0.001) was obtained between the two sets of CEA values. (Abbott RIA values were multiplied by 2, to correct for dilution.)

In general, we find the values obtained with either assay to be comparable, but for values exceeding 15 μg/Liter the Abbott CEA assay gives a higher value than that obtained with the Roche CEA assay because the latter gives a nearly flat curve in this region. The new test requires about 3 h of technician time as compared to 6 h with the Roche assay, but the total time required for the assay is the same because of the longer incubation required in the solid-phase assay.

In addition, the Abbott CEA test gives the advantage of eliminating dilution and errors due to fluctuation in pH and ionic strength, which are the main drawbacks of the Roche CEA assay.

References

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More on Serum Enzymes in Cancer Patients

To the Editor:
In a recent issue of Clinical Chemistry (23, 2034 (1977)) a study appeared on activities of 5-nucleotidase (5-NT), γ-glutamyltranspeptidase (GGT), alkaline phosphatase (AP), and glutamate dehydrogenase (GLDH) in sera of cancer patients. This paper quotes our earlier work on this subject (1) but later papers (2, 3) dealing with these and other enzymes in cancer patients are not discussed. Obviously, the reason is that our work, well known in Europe, was published in a journal not generally found in American hospital libraries. We think it would be useful to summarize the results of our work for your readers. A review may help to preclude work already published being duplicated without comparison of the earlier results. A well-documented description of patients and materials will be found in the references listed below.

We were first to report a method for measuring serum 5-NT in which adenosine daminase is used to generate NH₃ from adenosine during incubation. The NH₃ is measured by the indophenol reaction of Berthelot (4), which terminates the incubation without deproteinization.

In a following paper (5) the addition of phenyl phosphate as a successful inhibitor of the apparent nucleotidase effect of bone phosphatase was described, and later several conditions affecting the assay using adenosine deaminase were amply discussed. In 1969 we undertook comparative studies on AP—with special reference to bone phosphatase—5-NT, alanine aminotransferase, and aspartate aminotransferase (2) in the follow-up of cancer patients during various kinds of therapy. The study was continued until 1974, including GGT in another series of patients, making a total of more than 1000 subjects involved.

An important point in such studies is that serum enzymes in cancer patients