Tissue LDH isoenzyme Assays in the Detection of Colonic Carcinoma

To the Editor:

Readily available methods for detecting early or pre-malignant conditions are needed. LDH isoenzyme assays have been recommended in detecting early colonic carcinoma.

We have investigated the value of measuring LDH isoenzymes in colonic and rectal mucosal tissue. In normal colonic tissue LDH isoenzymes 1 and 2 predominate, whereas in malignant tissue there is a relative excess of LDH isoenzyme 4 and 5, which results in an increased LDH isoenzyme (4 + 5)/1 + 2 ratio. These LDH isoenzyme pattern shifts have been reported in morphologically uninvolved tissue surrounding the neoplasm, and it has been suggested (1, 2) that evaluating alterations in the LDH isoenzyme pattern may be of use in the early detection of cancer.

We have assayed LDH isoenzyme (4 + 5)/(1 + 2) ratios in 21 rectal biopsies from a control group (60 men, 15 women; age range 21–80 years, median age 44 years) who had sigmoidoscopy at a gastrointestinal outpatient clinic. All of these patients were subsequently found to be free of colonic disease. LDH isoenzyme ratios were also determined in surgical biopsies of malignant tissue and in biopsies of uninvolved tissue surrounding the neoplasm. All biopsy specimens were stored at −70 °C until analysis within a week. The isoenzymes were analyzed by use of the Corning agarose gel electrophoretic method, in which an AMP–lactate–sucrose substrate coenzyme solution and fluorometric detection are used.

Our results (Table 1) do not reveal any change in the LDH isoenzyme ratios in the mucosa of the aging colon. No significant difference was found between the isoenzyme ratios in the control patients and the carcinoma patients, either in the actual carcinoma biopsies or in the surrounding tissue. Therefore, we conclude that, in our hands, the measurement of LDH isoenzyme ratios has no predictive value in the early detection of colonic carcinoma.

References


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Table 1. Results for Controls and Colonic Carcinoma Patients

<table>
<thead>
<tr>
<th>Subjects</th>
<th>LDH isoenzyme ratio</th>
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</thead>
<tbody>
<tr>
<td>Controls</td>
<td>(4 + 5)/(1 + 2)</td>
</tr>
<tr>
<td>21–39 yrs (n = 9)</td>
<td>2.8 ± 0.7</td>
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<tr>
<td>40–59 yrs (n = 5)</td>
<td>4.2 ± 1.4</td>
</tr>
<tr>
<td>60–80 yrs (n = 7)</td>
<td>3.6 ± 1.1</td>
</tr>
<tr>
<td>Carcinoma patients (n = 6)</td>
<td></td>
</tr>
<tr>
<td>Uninvolved mucosa</td>
<td>3.1–1.0</td>
</tr>
<tr>
<td>Carcinoma</td>
<td>4.5–1.8</td>
</tr>
</tbody>
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To the Editor:

The binding of Coomassie Brilliant Blue (CBB) to proteins shifts its absorption maximum from 450 to 600 nm. This property was first used by Bradford (1) for the quantitative determination of proteins in biological fluids. Sedmak and Grosberg (2) showed that the ratio of absorbances at 620 and 465 nm is a linear function of protein concentration. Commercial reagent kits and apparatus for protein assay in urine and cerebrospinal fluid by CBB binding are now on the market.

We recently tested the "Proti-analizer" manufactured by Marius, Utrecht, The Netherlands. The photometer is especially designed for measuring the ratio of absorbances at 595 and 465 nm. After adjustment with a blank and a protein standard, results for the ratio of absorbances are converted into protein concentration. The reagent supplied by the manufacturer of the colorimeter consists of 98 mg of CBB G250, 49 mL of methanol, 98 mL of phosphoric acid, and 20 mL of acetic acid brought to 1-L water. The photometric readings are made 10 min after mixing 10 mL of centrifuged urine with 2 mL of reagent.

For standards, we used Labtrol-E (Merz + Dade AG, Düdingen, Switzerland), diluted with isotonic saline (NaCl 150 mmol/L). 1, 2, 3, or 4 g/L. The photometric response to standards is linear up to 4 g/L. The method shows good reproducibility with standards or with urine samples (CV = 3% at 1 g/L).

We compared the CBB method with another dye-binding method (3) in which proteins are precipitated with 30 g/L trichloroacetic acid (TCA) in the presence of Ponceau Red (PR), and the amount of dye bound to precipitated proteins is measured. We introduced a slight modification of the procedure to avoid drug interference (4).

Centrifuged urine samples, selected to cover a wide range of protein concentrations (0 to 3 g/L), were analyzed by both methods. The results correlated fairly well (r = 0.977, n = 84), but the regression line deviated significantly both from the origin and from 45° (y = 0.19 + 0.86x, where y = proteins by CBB and x = proteins by PR). The differences in protein composition of Labtrol and urine, and the difference in affinity for the two dyes, probably explain why the regression coefficient is not 1 and why the CBB estimate is lower than the PR estimate at high protein concentrations.

However, for most urine samples of low protein concentration (less than 0.3 g/L by PR), CBB gave higher values (by 0.2 g/L on the average), and the results of the two methods at these concentrations were not correlated at all (r = −0.09, n = 19). These observations suggest that CBB reacts with urine components that do not bind PR. Suspecting that mucoproteins were responsible for the discrepancy, we selected 20 centrifuged urine samples of low protein content (less than 0.3 g/L by PR), and precipitated their proteins

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with either TCA or perchloric acid and acetone (PCA-A) (5). After centrifugation, the precipitates were dissolved in a small amount of NaOH, 0.5 M/L, and their protein content was measured by the CBB method. Prior precipitation standards (diluted Labtrol), with TCA or PCA-A did not modify the binding capacity of proteins to CBB.

Applying the CBB method to urine proteins precipitated by TCA gave results in good agreement with the PR method, the average concentration of protein being 123 and 116 mg/L, respectively (r = 0.86). Moreover, all CBB-reacting material of urine was precipitated by PCA-A: the respective average concentrations of protein were 355 and 353 mg/L (r = 0.97). Obviously, the discrepancy between CBB and PR is due to a urine component that binds CBB and is not precipitated by TCA. The average difference was 240 mg/L (range, 0 to 610 mg/L).

Mucoproteins are soluble in TCA, and the main urinary mucoprotein, Tamm–Horsfall protein, binds CBB as other proteins do (6). Thus these urine components are probably responsible for the differences in apparent proteinuria measured by the two methods. Although mucoproteins are expected to be always present in urine (7), a variable part of them may be discarded with the sediment before the chemical analysis of urine (8). Saifer and Gerstenfeld (5) found a highly variable amount of TCA-soluble proteins, ranging from 0 to 0.5 g/L. Our results are consistent with these observations.

Because the CBB method measures mucoproteins as well as other proteins, it gives a more exact estimate of the total amount of urinary proteins; however, this very improvement decreases the diagnostic specificity of proteinuria. The upper limit of physiological proteinuria measured by turbidimetry with sulfosalicylic acid or by TCA precipitation is below 0.2 g/L for average urinary flow (9). With the CBB method, a variable amount (up to 0.6 g/L) of mucoproteins is included in the result, so that the threshold of pathological proteinuria becomes very imprecise.

Laboratories using CBB for determination of urinary protein should be aware of this drawback and warn the clinicians that the results are not equivalent to those of conventional methods for low proteinuria.

References

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Isolation of DNA from Biological Specimens without Extraction with Phenol

To the Editor:

In isolating DNA from biological specimens (blood, leukocytes, tissue, urine and cultured cells), distilled phenol is usually used to extract proteins and other material that may interfere with subsequent analysis for DNA. Distillation of phenol is not a practical procedure for most clinical laboratories, and although commercial supplies of relatively pure phenol are available, its shelf life is limited because of the formation of phenolic free radicals, which can cross-link DNA and cause anomalous results. More importantly, the use of such a noxious reagent, which has several associated safety hazards, is undesirable for a clinical laboratory setting.

DNA studies for genetic and infectious disease applications will become a valuable tool for the clinical laboratory in the foreseeable future. Two requirements for such studies are: (a) simple and rapid methods for isolation of genomic DNA, which can then be manipulated by using restriction endonucleases, and (b) nontoxic labels for DNA and RNA probes.

We report here a modification of a standard DNA isolation procedure (1) that does not involve phenol extraction. We have applied this modified procedure to nucleated cells from peripheral blood and cultured cell lines; however, the procedure should be applicable to tissue pieces as well.

For peripheral blood leukocytes: Collect 20 mL of heparinized blood in a 50-mL conical tube. Separate plasma and cells by centrifugation (1800 × g, 20 min) at ambient temperature. Aspirate the plasma to approximately 5 mm above the buffy coat. Add the lysis buffer to the cell pellet to give a final volume of 50 mL (lysis buffer: 0.32 mol of sucrose, 10 mmol of Tris HCl, pH 7.5, 5 mmol of MgCl2, and 10 mL of Triton X-100 per liter). Mix the suspension by inversion and allow it to stand in an ice bath for 30 min.

Centrifuge the suspension for 15 min at 1800 × g and remove 40 mL of the supernate (the nuclear pellet will not be visible, owing to the high concentrations of hemoglobin in the supernate). Resuspend the pellet in lysis buffer to a final volume of 50 mL and recover the nuclear pellet by centrifugation.

For cultured cells: Wash the cells in Hank’s balanced salt solution (Gibco Laboratories, Grand Island, NY 14072), then prepare the cell pellet by centrifugation (1800 × g, 15 min). Add lysis buffer to a final volume of 50 mL, mix by inversion, and leave in an ice bath for 30 min. Recover the nuclear pellet by centrifugation at 1800 × g for 15 min.

Regardless of the sample type, remove and discard the final supernate. Resuspend the pellet from peripheral blood or cultured cells in 5 mL of a solution containing 200 mmol of Tris HCl, pH 8.5, 100 mmol of EDTA, and 35 mmol of sodium dodecyl sulfate per liter. Avoid vigorous agitation, which may break up the DNA. Add 100 μL of a 10 mg/mL solution of Proteinase K (Boehringer Mannheim, Indianapolis, IN 46250) dissolved in de-ionized water, then incubate the suspension for 4 h at 60 °C. Add an additional 100-μL aliquot of Proteinase K and continue the incubation overnight at 37 °C. The following morning, add 1 mL of 6 mol/L NaClO4 and incubate for 2 h at 37 °C. Cool the suspension to ambient temperature and add 1.5 mL of 5 mol/L potassium acetate. Mix gently to obtain a homogeneous suspension and then let stand for 30 min in an ice bath. Centrifuge the suspension (27 000 × g, 15 min, 2 °C). Decant the supernatant fluid into a separate tube and discard the pellet. Precipitate the DNA by adding two volumes of ethanol. Let the solution stand for 15 to 30 min and, in the case of mammalian genomic DNA, harvest the DNA by "winding" the DNA onto a glass rod. Remove the ethanol by evaporation and resuspend