Plasma Malondialdehyde, Lipid Peroxides, and the Thiobarbituric Acid Reaction

To the Editor:

We read with interest the Letter by Hackett and her colleagues (1) concerning use of the thiobarbituric acid (TBA) test to measure plasma malondialdehyde (MDA), an indicator of in vivo lipid peroxidation. They present data showing that this commonly used test is neither specific for plasma MDA nor reproducible. They also observed that analytical recovery of MDA added to plasma samples was poor.

We agree with them that the fluorimetric method and indeed many other methods reported in the literature for measuring MDA-TBA are not specific or reproducible, but we believe this is because the TBA test has been inappropriately applied to biological samples. Furthermore, plasma TBA reactivity has not been adequately characterized or standardized, resulting in many misconceptions as to what is actually being measured by the TBA test.

The TBA test was initially proposed as a sensitive method for quantifying MDA, one of the many secondary products formed during lipid peroxidation. Relatively simple to perform, it involves heating samples under acidic conditions with TBA, which reacts with the MDA to form a red complex (MDA-TBA), which in turn is measured spectrophotometrically at 532 nm, or by fluorimetry.

Many workers commonly use this "simple" test to measure free-radical-mediated lipid peroxidation in complex biological tissues and fluids. However, recent studies have shown that the TBA reaction is notoriously nonspecific, because non-lipid compounds commonly present in biological samples—including carbohydrates, pyrimidines, and hemoglobin—also react with TBA (2–4), forming colors that have spectra overlapping that of authentic MDA-TBA, interfering with measurements at 532 nm. Furthermore, it is now well established that TBA not only reacts with "free MDA" but also with MDA that is formed from metal-catalyzed thermal decomposition of lipid peroxides. The TBA reactivity of these peroxides is strongly influenced by the type of acid used and the presence of metal ions and peroxyl radicals, as well as the method of sample preparation (3). Unfortunately, problems associated with the TBA specificity and reactivity have been largely ignored by many workers, who have adopted their own versions of the method. This makes virtually impossible the interpretation and comparison of the many publications on plasma lipid peroxides in health (Table 1) and disease states.

In our laboratory we have developed an HPLC method that eliminates nonspecific problems from the TBA assay (2, 3). Using this technique, we established reaction conditions under which MDA-TBA formation is quantitative. Our observations suggest that Fe must be present if conversion of the peroxides to MDA is to be optimal, and second, that the presence of antioxidants is essential to prevent in vitro peroxidation of other unsaturated fatty acids. We also investigated the source of TBA reactivity in plasma samples (3).

Not surprisingly, our observations show clearly that whole-plasma samples cannot be used for MDA-TBA measurements because of artifactual MDA arising from non-lipid sources. This problem is overcome by using lipid extracts of plasma, which eliminate potentially interfering water-soluble substances such as carbohydrates. Autodissociation of lipids during the test along with artifactual MDA estimations resulting from overlapping spectra and non-lipid-derived MDA are, we believe, responsible for the large variations in reported plasma MDA values. Perhaps our attempts to characterize and standardize what has been a much-abused and inappropriately applied assay will result in a more meaningful assessment of the role of lipid peroxidation in health and disease.

References

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Table 1. Reported Lipid Peroxide Concentrations (as MDA-TBA) in the Plasma or Serum of Healthy Subjects

<table>
<thead>
<tr>
<th>Year</th>
<th>Technique</th>
<th>Lipid peroxides, mean (±SD), pmol/L</th>
<th>Ref. no.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1983</td>
<td>Spectrophotometry</td>
<td>47.2 (7.0)</td>
<td>4</td>
</tr>
<tr>
<td>1980</td>
<td>Spectrophotometry</td>
<td>35.1 (5.9)</td>
<td>5</td>
</tr>
<tr>
<td>1983</td>
<td>Spectrophotometry</td>
<td>6.6 (5.9)</td>
<td>3</td>
</tr>
<tr>
<td>1986</td>
<td>Spectrophotometry</td>
<td>4.2 (0.7)</td>
<td>7</td>
</tr>
<tr>
<td>1981</td>
<td>Fluorimetry</td>
<td>3.2 (0.9)</td>
<td>4</td>
</tr>
<tr>
<td>1984</td>
<td>Fluorimetry</td>
<td>1.7 (0.5)</td>
<td>4</td>
</tr>
<tr>
<td>1988</td>
<td>HPLC</td>
<td>1.4 (0.3)</td>
<td>3</td>
</tr>
<tr>
<td>1986</td>
<td>Spectrophotometry</td>
<td>0.9 (0.1)</td>
<td>4</td>
</tr>
<tr>
<td>1987</td>
<td>HPLC</td>
<td>0.5 (0.1)</td>
<td>4</td>
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</table>

Nonprotein Components of Urine Interfere with Colorimetry of Urinary Albumin with Bromphenol Blue

To the Editor:

Determination of albumin in urine is important for follow-up of diabetic patients, because microalbuminuria is a strong predictor of the development of diabetic nephropathy. The method for measuring microalbuminuria must be sufficiently sensitive to determine urinary albumin between 10 and 200 mg/L, concentrations not detectable with dipsticks. Various techniques (e.g., radioimmunoassays, enzyme immunoassays, immunoturbidimetry, and im-
When we consider that creatinine and uric acid, for example, are excreted in concentrations of about 15 and 5 mmol/L, we can already observe positive biases caused by these interferences at a sample:reagent volume ratio of 1:5 (1). However, this ratio does not yet permit measurement of microalbuminuria. Thus, the interfering reactions of urinary components with bromphenol blue preclude use of this dye-binding method for determination of microalbuminuria in native urine samples. These interferences by non-protein constituents of urine would explain why the Microalbutest (Miles Labs., Ames Div.) showed falsely positive results (2).

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More on Oligoclonal Bands and Diagnosis of Multiple Sclerosis

To the Editor:

We would like to report our contribution regarding the "dilemma" raised by Stanley et al. (1) and recently discussed by Tate and Campbell (2), concerning the relationship between the diagnosis of multiple sclerosis (MS) and the presence of oligoclonal bands that are unique to the cerebrospinal fluid (CSF).

We considered isoelectric focusing (IEF) patterns for CSF and serum from 94 patients, who met the criteria of McDonald and Halliday (3) for a clinical diagnosis of definite MS. The specimens, collected between January 1985 and July 1986, were all treated in the same way. Essentially, we standardized the concentration of IgG in CSF (by using Amicon CS 15 concentrators) and in serum (by diluting the sera with distilled water) so we could apply a constant amount, 20 μg, of IgG to the gel surface by use of pieces of filter paper. For the IEF we used thin-layer polyacrylamide gel (Ampholine PAG-plate, pH range 3.5–9.5; LKB), staining with Coomassie Brilliant Blue.

On the basis of the IEF findings, the 94 patients could be divided into three groups. Group I, comprising 66 patients (70%), had oligoclonal bands, numbering three or more, only in their CSF. Group II, 20 patients (21%), had some bands common to both CSF and serum but also some unique to CSF. Group III, eight patients (9%), had no oligoclonal bands in either CSF or in serum. Thus Groups I and II, with at least three or more bands in CSF only, represented 91% of all these patients.

One patient affected by both MS and, in association, Sjögren's syndrome (specific antibodies to SSA/Ro present in his serum) had multiple oligoclonal bands in CSF and also three that appeared in IEF patterns for both CSF and serum. This might result from the combination of two different immunological mechanisms, functioning in the systemic and intrathecal compartments, respectively.

We also found bands unique to CSF, together with some others paired in CSF and in serum, in 78% (18 of 23) cases of MS with central nervous system involvement.

In a group of other neurological diseases (e.g., extrapyramidal degenerative disease, neurosyphilis, chronic myelopathy of unknown cause, polyneuropathy, Guillain–Barre syndrome, and dementia) we found some cases that showed, on IEF, patterns of bands identical in CSF and serum and, occasionally (<5%), bands unique to CSF. Moreover, our preliminary data concerning patients with acute unilateral optic neuritis indicate that those patients who have oligoclonal banding in CSF have an increased risk for future development of MS. This underlines the importance of detecting oligoclonal fractions in CSF from cases of suspected MS, as an aid in early diagnosis of the disease.

In any case, in agreement with Tate and Campbell (2), we never found any patient with clinically definite MS who had an identical banding pattern in both CSF and serum.

References


2. Tate J, Campbell B. Must CSF oligoclonal bands be unique to cerebrospinal fluid and absent from serum to support the