A Better Method for Eliminating Salicylate Interference with Measurement of Acetaminophen

To the Editor:

Acetaminophen is available in many proprietary combinations with aspirin (acetylsalicyclic acid) and salicylamides (1, 2), so any method for acetaminophen measurement should be free from interference by aspirin and its related compounds. Reed et al. (3) recently reported that a commercial kit method for acetaminophen analysis ("Rapid Stat Kit", Lancer Division of Sherwood Medical, St. Louis, MO 63101) suffers from a significantly stronger positive interference by salicylate than was indicated in the package insert.

Salicylamide, the drug most commonly combined with acetaminophen in "aspirin-free" analgesics, is readily hydrolyzed to salicylate. Both salicylamide and salicylate interfere positively with nitration methods (4).

A direct acid/ferric reduction method for acetaminophen that is free of interference by salicylate and salicylamides was reported earlier by Liu and Oka (5). In this method a complex of ferric 2,3,6-tris-(2-pyridyl)-S-triazine is reduced to the ferrous complex by the phenolic hydroxyl group of acetaminophen. Aspirin does not interfere because its hydroxyl group is acetylated and therefore the compound does not have a phenolic hydrogen to donate as an active reducing agent Table 1. The potential reducing hydrogens of the phenolic groups of salicylate and salicylamides are both stabilized by internal hydrogen bonding to the oxygens of the carboxyl and amide groups, respectively. These stabilizations explain the minimal interference observed with this method for acetaminophen (Table 1). In the therapeutic concentration range for salicylate and salicylamides (<50 mg/L) in serum, neither reagent interferes with the method, and even at toxic concentrations (600–1000 mg/L) the three nitratable aspirin-related compounds yield negligible positive interference.

Moreover, the method is rapid (approximately 15 min with the manual technique); involves stable, safe reagents; and is comparatively free of interferences. Thus, we recommend it for consideration by laboratories that analyze for acetaminophen.

References

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Creatine Kinase B-Subunit Activity in Serum in Cases of Suspected Myocardial Infarction: A Prediction Model Based on the Slope of MB Increase and Percentage CK-MB Activity

To the Editor:

Assay for creatine kinase (CK; EC 2.7.3.2) MB isoenzyme by immunological inhibition of the M-subunit activity and measurement of the residual B-subunit activity has been extensively evaluated (1–3). This method is simple, fast, and precise, but the requirements for quantitative accuracy, sequential testing, and the recognition of subendocardial infarcts (4) and infarct extension compelled a further investigation in our laboratory.

To minimize errors of conventional diagnostic cutoff values and other procedures that do not optimize for time-dependent changes and variability of the measured results, we utilized receiver operator characteristic curves (5, 6) for nonoverlapping time intervals during the course of infarction, noting

Table 1. Effect of Salicylate, Salicylamide, and Aspirin on Measurement of Acetaminophen by the Direct Acid/Ferric Reduction Method

<table>
<thead>
<tr>
<th>Conc, mg/L</th>
<th>Apparent acetaminophen, mg/L</th>
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<tbody>
<tr>
<td>Salicylate</td>
<td></td>
</tr>
<tr>
<td>&lt;50*</td>
<td>0</td>
</tr>
<tr>
<td>100</td>
<td>0.7</td>
</tr>
<tr>
<td>300</td>
<td>3.5</td>
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<tr>
<td>600</td>
<td>7.6</td>
</tr>
<tr>
<td>1000</td>
<td>15.0</td>
</tr>
<tr>
<td>Salicylamide</td>
<td></td>
</tr>
<tr>
<td>&lt;50*</td>
<td>0</td>
</tr>
<tr>
<td>100</td>
<td>1.1</td>
</tr>
<tr>
<td>300</td>
<td>3.3</td>
</tr>
<tr>
<td>600</td>
<td>6.7</td>
</tr>
<tr>
<td>Acetylsalicylic acid</td>
<td></td>
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<td>1000</td>
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*Within therapeutic range; all others listed are toxic concentrations.
the ascent, the peak, and the decline of the CK and CK-MB values in the different groups of patients. As a result of our serial studies of CK activity and CK-MB determinations at 6-h intervals after admission to determine what effect timed sampling may have on the diagnostic efficiency, we developed an empirical prediction model to separate patients into characteristic groups defined by the slope of CK-MB increase and the CK-MB fraction as percent of total CK activity.

We studied 66 patients suspected of having had a myocardial infarction (MI), who were admitted to the coronary-care units. Half of them subsequently were found to have MI as determined from the discharge medical records. The patients studied were admitted with clinical symptoms within 24 h of onset, and most had chest pain considered to be consistent with severe angina or myocardial infarction. In the absence of electrocardiographic changes indicative of MI, the presence of CK-MB fraction was used for the assessment and final diagnosis. We generated receiver operator curves for the admission, 6-h, and 12-h sampling times by determining the sensitivity and specifity of CK-MB activity in 2 U/L increments from 10 to 18 U/L, using the discharge diagnosis as the medical basis for classification. The values chosen for the times and the CK-MB activities used to construct the receiver operator curves were derived from an analysis of the serial changes in serum CK and CK-MB, determined at the time of admission and every 6 h for the first 24 h and daily for three additional days after admission in an initial survey involving 24 patients, 12 of whom were established to have had MI.

Serum CK activity was measured with an acu (Du Pont Instruments, Wilmington, DE 19898) or a KDA (American Monitor Corp., Indianapolis, IN 46226) analyzer, at 37 °C. The reference ranges for CK activity were the same for both instruments. Serum CK-MB activity was determined with an ABA-100 (Abbott Diagnostics, N. Chicago, IL 60064) discrete analyzer and with the BMC (Boehringer Mannheim Diagnostics, Indianapolis, IN 46250) kit, which measures immunoinhibition of the CK M-subunit at a reaction temperature of 37°C. The results obtained by measuring the residual CK B-subunit activity were not multiplied by the factor of 2. The discrimination limit for the B-subunit activity was 13 U/L. Qualitative and quantitative electrophoresis in agarose gel was used to identify the CK isoenzymes. We also measured lactate dehydrogenase isoenzyme 1 (LD-1) activity by "isomune" (Roche Diagnostics, Nutley, NJ 07110) whenever confirmation of increased LD-1 activity was required. All doubtful cases were studied by CK electrophoresis and LD-1 immunnoassay.

We found an increase in the CK-MB activity in the MI group of patients that was not demonstrable or significant in the non-MI group, with the ascent peaking at 10 to 15 h after admission. The mean of CK-MB activity was 6.7 U/L (SD 3.9 U/L) for all non-MI patients, with no significant difference among 0, 6, and 12-h time segments. The mean of CK-MB activity was 29.2, 57.4, and 83.3 U/L (SD 18.1, 49.4, and 58.8 U/L) over the same time periods, respectively. While the means of the CK-MB activities for the MI group increased almost linearly, the large increase observed in the variance by 6 h probably reflected the heterogeneity of patients with respect to infarct size and other uncontrollable factors. The 95% intervals for the MI patients at the time of admission represents 4.5-fold the CK-MB activity of the non-MI patients. The 95% intervals for the CK-MB activity in the group increases linearly ($r^2 = 0.9811$) with an intercept at 72.8 U/L and a slope of 11.3 U/L per hour over the 12-h period of increase. We noted that CK-MB disappears promptly 12 h post-admission and that the population variance at the time of admission is great enough to introduce an error in interpretation of a border-line increase. We also determined that the diagnostic efficiency exceeded 95% at a level of 14 U/L for all sampling times from receiver operator curves for the CK-MB with variable cutoffs taken at admission, 6 h and 12 h.

As we gained greater experience with the method, it became increasingly clear that there was a potential for confusion in up to 10% of intensive-care patients when we used a cutoff value of 14 U of CK-MB per liter, because of patients with massive trauma, occasional alcoholics, patients with chronic lung disease, peri-operative patients, or those who had increased total CK activity disproportionately greater than the increase in CK-MB. Such patients with noncardiac CK-MB increases usually had 5% CK-MB, whereas patients with uncomplicated MI had a CK-MB increase that was 6 to 8% of the total CK activity. Further, the presence of CK-BB isoenzyme activity could be identified by using the observation of CK-MB activity with constant activity during the sampling period.

We pursued several applications of combinations of variables that could be used as optimum predictors of patients with and without MI in a set of predictors based on the fact that the patients with MI usually have more than 6% CK-MB and a slope of CK-MB increase >0.8. Figure 1 illustrates that the MI group is segregated from the non-MI group by using the combined variables as predictors. All of the MI patients have a slope of CK-MB increase >0.9. All of the patients in whom MI is excluded have CK-MB that is <6% of the total CK activity and, except for one instance, have a slope of CK-MB increase that is <0.8. Although most patients with MI have CK-MB >6% of the total CK activity, there are six patients with CK-MB <6% of the total CK activity. This represents a significant population with CK activity that is a mixture of myocardial and noncardiac-derived CK. Nevertheless, the patients who clearly have MI and those who can be excluded may be readily identified by whether they fall in a zone defined by CK-MB >5% of total CK activity and slope of CK-MB >0.8 or defined by CK-MB <6% and slope of CK-MB increase <0.8 as coordinates for a two-variable matrix. Some of the unclassified patients in Figure 1 had CK-MB of 6% or more with no increase of the CK-MB slope. This group included patients who had MI and were on the downslope of the CK-MB decay curve.

We conclude that measurement of CK-MB at two or more times during the appearance phase after admission is very valuable to determine whether CK-MB is increasing or decreasing. Moreover, the frequency with which CK-MB appears at or above the diagnostically significant level in patients in whom the diagnosis of MI cannot be proven or excluded is significant. This problem is addressed by the present study.

References
2. Gerhardt W, Waldenstrom J: Creatine kinase B-subunit activity in serum after

Fig. 1. Distribution plot of slope of CK-MB increase (ordinate) against percent CK-MB (abscissa) in patients with myocardial infarction (open circles), nonmyocardial infarction (open squares), and unclassified (usually late myocardial infarction) (open triangles)

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Sulfasalazine Interference in Total Protein Measurements with the Du Pont aca

To the Editor:

Sulfasalazine (Azulfidine; Pharma- cia) is commonly used to treat mild to moderate ulcerative colitis, Crohn's disease, or an adjuvant agent for patients with severe ulcerative colitis. After ingestion, about a third of the dose of sulfasalazine is absorbed from the small intestine; the remainder is metabolized to sulfapyridine and 5-aminosalicylic acid. Little of the 5-aminosalicylic acid is absorbed; most is excreted unchanged. Nearly all the sulfapyridine, however, is absorbed from the colon and later excreted in the urine.

We recently received a request to measure albumin and total protein in serum from a 55-year-old man with severe ulcerative colitis, who was receiving 8 g of sulfasalazine per day. We used a Du Pont Automatic Clinical Analyzer (aca) for these assays, the results being as follows: albumin, 24 g/L, total serum protein, 24 g/L. Although the Du Pont aca manual states that total serum protein measurements may be falsely depressed when patients are taking sulfasalazine, no literature citation or suggested reference method free of such interference is provided. Believing that sulfasalazine (or its metabolites) was the source of interference here, we measured total protein and albumin by four different methods. We used a control serum (Monitrol I and Monitrol II; Dade, Miami, FL 33152) to which we had added sulfasalazine or sulfapyridine. Table 1 shows the results.

The aca method for total protein is an endpoint biuret reaction with readings at 510 and 540 nm. Because we thought that the spurious low results for total protein we had noted were ascribable to over-blanking, we examined the absorbance spectra of sera with and without added sulfasalazine. Prior to the biuret reaction, there was little difference in the absorbance of serum at 510 or 540 nm when sulfasalazine was added. We also examined the absorbance spectrum of the fluid remaining in the aca pack after completing the total protein assay for sera with and without sulfasalazine. There was a substantial increase in absorbance at 510 nm for the serum containing sulfasalazine as compared with the control serum. Thus we conclude that a major cause of sulfasalazine interference with the aca method for total protein measurements is an "over-blanking" related to an increase in absorbance at 510 nm after biuret reaction completion.

Evidently, patients receiving sulfasa- lazine should have total serum pro-
tein measured by a manual biuret pro-
cedure with a serum blank rather than by the aca.

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Alternative Approach to Two-
Dimensional Gel Electrophoresis of Serum

To the Editor:

High-resolution, two-dimensional electrophoresis (1), now widely used to examine the proteins of human serum (2–4), involves denaturing the proteins (with mercaptoethanol and urea), then isoelectric focusing (IEF) in the presence of urea (9 mol/L) and sodium dodecyl sulfate/polyacrylamide gel electrophoresis (SDS-PAGE).

After dissociation into their constituent polypeptides, individual proteins are often resolved into multiple spots, which can hinder their identification. This problem can be partly overcome by adopting a low-resolution two-di-
dimensional approach involving electrophoresis of the native proteins in agarose gels before SDS-PAGE, in the absence of reducing agents (5). This procedure resolves about 60 plasma proteins, most of them as single spots (5). I have investigated a similar ap-
proach but have used IEF in polyacryl-
amide gels to separate the native pro-
teins before SDS-PAGE without added reducing agents. This alternative approach resolves the proteins of human serum into at least 120 spots.

The procedure used for two-dimen-
sional electrophoresis was similar to that previously described (3), but the composition of the polyacrylamide-gel cylinders (IEF) was modified by omit-
ting the 9.0 mol/L urea and substitut-

| Table 1. Effect of Sulfasalazine and Sulfapyridine on aca Protein Measurements |
|---------------------------------|---|---|---|---|
|                               | I  | II | I  | II |
| Control serum                 | 66 | 51 | 62 | 52 |
| Control serum plus sulfasalazine, 50 mg/L | 49 | 25 | 67 | 57 |
| Control serum plus sulfapyridine, 80 mg/L | 66 | 51 | 68 | 59 |

* Du Pont aca.
\textsuperscript{b} Turbidimetric (Du Pont method for cerebrospinal fluid protein after 100-fold dilution of serum).
\textsuperscript{c} Manual biuret, reagent blank.
\textsuperscript{d} Manual biuret, serum blank.
\textsuperscript{e} I and II refer to Monitrol I and Monitrol II, respectively.

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