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To the Editors:

We thank Chappell et al. for bringing to our attention their brief communication regarding HPLC separation of angiotensins [1]. We were unaware of this work. Reference to their HPLC method was not included in any of the method publications that we reviewed. Furthermore, three separate computer-based literature searches failed to identify the principal article describing their assay. One possible explanation was that we focused our literature review on HPLC-RIs for Ang II in human plasma, and the references included in the letter by Chappell et al. are from investigations in nonhuman models, specifically dog, rat, and vascular smooth muscle cells. Recognizing the difficulty in familiarizing ourselves with all publications from the extensive literature pertaining to Ang II and related peptides, and the possibility that pertinent information may have escaped our review, we qualified the statement in our manuscript about the novelty of using a mobile phase containing HFBA to separate angiotensins.

Even though our chromatographic method [2] is similar to that described by Chappell et al. there are several important differences. Our HPLC conditions were optimized specifically for Ang II in human plasma, so that this peptide could be collected and analyzed in a single fraction. Because accurate quantification of Ang II requires reproducible elution and because peptide elution is highly sensitive to small changes in the amount of organic modifier, we developed an isocratic procedure with a washing and reequilibration step between injections. In contrast, the method by Chappell et al. involves gradient elution conditions in which the peptides are collected in multiple fractions. The collection and assay of multiple fractions is more labor intensive, time consuming, and costly. We did not demonstrate separation of Ang II from the same number of related peptides as Chappell et al. [3], because our goal was to resolve Ang II from peptides that cross-reacted with our anti-Ang II antibody. Thus, our investigation focused on separating Ang II from Ang 2–8 (Ang III), Ang 3–8, and Ang 4–8.

We regret that we failed to uncover and acknowledge the work by Chappell et al.; however, the results of both groups independently confirm the usefulness of HFBA as an ion-pair reagent for chromatographic separation of angiotensins. Future publications by our group concerning Ang II will include reference to the work by these investigators.

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Rare Interference in Determinations of Total Creatine Kinase

To the Editor:

Total creatine kinase (CK) from skeletal muscle may be a cause of error when released into the circulation (e.g., in cases of peripheral myopathy), where its activity contributes to that of total serum CK determined in patients with ischemic heart disease. However, and though extremely rare, total skeletal muscle CK activity may be added to total serum CK within the actual blood-collecting tubes if, after collection of the blood sample, muscle tissue is aspirated through the needle because of the negative pressure of the evacuated collection tubes used to extract the blood. This phenomenon occurred in the case we describe.

Our Emergency Laboratory was requested to determine the total CK and CK-MB in the evaluation of a patient tentatively diagnosed with acute myocardial infarction. The values of previous tests performed 6 h earlier were within the normal range for both analytes. The new assay (Tube 1) yielded CK of 2958 U/L (normal range, ≤200 U/L) and a CK-MB concentration of 36 U/L (normal range, ≤10 U/L), which represented 1.2% of the total CK. However, another tube of blood obtained at the same time (Tube 2) gave strictly normal values.

The results of the two tubes (in U/L) were as follows: Tube 1, CK 2958, lactate dehydrogenase (LD) 680, aspartate aminotransferase (AST) 147, CK-MB 36; Tube 2, CK 34, LD 427, AST 117, CK-MB 14. The rest of the biochemical analytes gave analogous results in both tubes. Samples collected later for assay of CK and CK-MB likewise yielded normal values.

Having excluded acute myocardial infarction in view of the clinical course and complementary explorations, and taking into account that only one tube exhibited abnormal results, we hemolyzed with distilled water all the blood in the sample tube that had given abnormal results (clotting had been prevented with lithium heparin); a remaining particle was detected that histologically corresponded mostly to skeletal muscle, connective tissue, and fat. This suggested that the tissue had entered the tube through the needle, drawn in by the reduced pressure in the sampling tube.

Experimentally, we also found that a 7-mg fragment of muscle 1 mm in diam-

References

eter added to 4 mL of blood with normal activities for total CK, LD, and AST yielded respective enzyme concentrations of 6070, 675, and 65 U/L (normal range ≤200, ≤440, and ≤65, respectively). The above finding should therefore be taken into consideration as a possible cause of a clinically unexplained increase in total CK.

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Influence of Dopamine on Peroxidase-Based Assays

To the Editor:

During the investigation of discrepant laboratory results, we detected an unusual interference in several of the biochemical tests. The patient's specimen had been obtained from a catheter in the jugular vein. Results of all analytes were discordant from those for a sample drawn 10 h previously. The cause of the discrepancy was thought to be contamination with isotonic saline containing 100 g/L dextrose. The dilution ratio of intravenous fluid to serum was ~1:10, as substantiated by several results obtained with an Ektachem 700 analyzer (Johnson & Johnson, Rochester, NY), including: sodium, 132 vs 140 mmol/L; potassium, 4.4 vs 4.8 mmol/L; chloride, 102 vs 112 mmol/L; and glucose, 21.8 vs 9.5 mmol/L. However, the creatinine result (96 vs 221 μmol/L) did not conform to the dilution ratio.

When the specimens were further analyzed with a Boehringer Mannheim/Hitachi 917 analyzer (Boehringer Mannheim, Laval, Quebec), and using the manufacturer's reagents, we noted a considerable decrease in the values for triglycerides (0.73 vs 1.23 mmol/L), cholesterol (1.3 vs 1.7 mmol/L), and uric acid (126 vs 528 μmol/L).

Upon investigation, we found that the sampling catheter was being used to administer dopamine at a rate required to maintain a steady-state plasma concentration of ~62 μg/L (renal dose). A review of the affected chemistries revealed that each of these analyses depended on the generation of peroxide for the indicator reaction, and that dopamine might be functioning as a reducing agent, resulting in the depletion of peroxide. To investigate our hypothesis, we analyzed three different sera with dopamine (or saline) added. Fig. 1 shows the mean decrease in analyte concentration. Control assays such as albumin and total protein, which do not rely on hydrogen peroxide, showed no interference (data not shown). All procedures were performed in accordance with the ethical standards of the institutional review board of the hospital.

As Fig. 1 shows, the interference starts at concentrations of dopamine ~1 mg/L. The steady-state concentration of dopamine in plasma is ~0.11 mg/L when administered for its cardiac inotropic effects and about one-half of that when given for renal vascular effects [1]. At these concentrations, dopamine does not appear to affect hydrogen peroxide-based assays. The extent of the interference appears to vary, depending on the amount of hydrogen peroxide generated in the initial reaction. Weber and van Zanten [2] have previously noted that dopamine has a negative influence on the enzymatic creatinine assay. To our knowledge, there have been no other investigations of this interferent.

Although the practice of obtaining blood specimens from intravenous lines is not ideal, it is convenient. We recommend that blood should not be drawn from intravenous lines when dopamine is being infused. Relatively small quantities of dopamine may affect assays that rely on hydrogen peroxide generation for their indicator reaction.

References


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