to be avoided. Berthod and Rey (1) have also reported enormous cross-reactivity of hydrocortisone hemisuccinate in the "Rianen" RIA kit for cortisol determination.

An issue of Clinical Chemistry (1983;29:890–953) reports the commercial availability of (then) more than 27 cortisol RIA kits. Methodologically, these kits were divided into three groups according to the different antibodies used: antibodies against cortisol-21-HS–BSA, antibodies against prednisolone-3-CMO–BSA, and antibodies against cortisol-6-hemisuccinate–BSA (2, 3). The specificity for cortisol depends on the specificity of the antibodies used. Briefly, in the case described above, kits in which antibodies against prednisolone-3-CMO–BSA and cortisol-6-HS–BSA are used showed a significant cross-reactivity for methylprednisolone and prednisolone (3). However, kits in which antibodies against cortisol-21-HS–BSA are used showed a significant cross-reactivity for deoxycortisol, 21-deoxycortisol, 17α-hydroxyprogesterone, and corticosterone (4). This consideration should always be borne in mind, because misleading results may ensue.

We are indebted to Dr. Ellen Ingham for encouragement, helpful discussions, and comments. We also thank Miss Misako Yagi for her excellent secretarial assistance and Miss H. Elliot for her help with English in the preparation of this manuscript.

This work was supported in part by the Japan Clinical Pathology Foundation for International Exchange and the Clinical Pathology Foundation of Japan.

More on "Hook Effects" in Immunoassays for Prostate-Specific Antigen

To the Editor:

Bodor et al. in their Letter (Clin Chem 1989;35:1262–3) and in their earlier paper (1) and also Alfthan and Stenman (2) discuss the occurrence of a "hook effect," observed while measuring serum prostate-specific antigen (PSA) by an immunoradiometric method (Tandem®-R PSA; Hybritech Incorporated, San Diego, CA 92126). They refer to the fact that in a simultaneous two-site immunometric assay samples with extremely high analyte concentrations may paradoxically produce a lower response than expected and on dilution produce a higher assay response, a phenomenon known as the "high-dose hook effect." Accurate quantification can ultimately be obtained by diluting the sample so that the sample response falls within the calibration curve of the assay. This effect is only of academic interest unless the undiluted specimen produces an analytical response below that of the highest calibrator. In general, immunometric assays are designed so that the highest expected value for patients will give a response higher than the response of the highest calibrator, thus indicating a need for dilution and avoiding a falsely low value.

Vaidya et al. (1) reported this effect in four of 721 samples; PSA concentrations were 650, 1840, 3280, and 6600 µg/L. Alfthan and Stenman (2) noted that of 332 sample runs undiluted and diluted, four at "<3000 µg/L" quantified at "<100 µg/L" when undiluted. Two others quantifying at 8600 and 8900 µg/L had a value of undiluted of <100 µg/L. These reports were surprising to us, because the Tandem-R PSA assay is designed—and every lot is tested—to ensure that a PSA concentration of 5000 µg/L will give an analytical response significantly higher than the response of the 100 µg/L calibrator. Three separate studies of the PSA "hook" profile obtained during three years, with use of purified PSA, suggested that the Tandem-R PSA method generally will produce a sample response exceeding the high calibrator until the PSA concentration well exceeds 10 000 µg/L (3). The highest concentration of PSA observed in our clinical studies was 7600 µg/L, observed for a Stage D prostate cancer patient.

Two of the four samples of Vaidya et al. (1) and four of the six samples of Alfthan and Stenman (2) did indeed have a response above the highest calibrator and therefore would have been diluted and accurately quantified. However, four of these 10 specimens unexpectedly read below the high calibrator. Early structural and functional studies of PSA suggest a possible explanation. PSA, a single polypeptide chain of 240 amino acid residues, is a proteolytic enzyme exhibiting both chymotrypsin-like and trypsin-like activity (4). It was observed that as much as 20% of purified PSA contained cleavages at three specific sites on the molecule, resulting in six identified fragment forms of PSA. These are believed to be a result of autocatalytic digestion of the PSA molecule at high concentrations. We likewise observed the degradation of a purified preparation of PSA stored at 4 °C into multiple species which were resolved by sodium dodecyl sulfate/polyacrylamide electrophoresis (3). In general, PSA at low to moderate concentrations seems to be relatively stable in serum (5). However, the stability of samples with extremely high PSA concentrations has not been studied. Possibly the proteolytic activity of moderately increased PSA is inhibited by various serum proteins, such as α1-antitrypsin. If so, in a serum with extremely high concentrations of PSA, this enzyme may be able to autodigest to immunologically active fragments that can bind to the capture or tracer antibodies but cannot form a "sandwich" and therefore will not be measured. High concentrations of binding fragments would inhibit the binding of the measured intact PSA molecule. Diluting such a sample would dilute out the inhibition, allowing proportionately more of the intact molecule to bind. The result could be the observation of an apparent hook effect at PSA concentrations lower than expected.

Extending the endoproteolytic cleavage observations of Watt et al. (4) from purified PSA to serum PSA has not been done. However, it is noteworthy that Alfthan and Stenman (2) found 9% of the serum PSA activity in one "hook" sample to reside in a 16-kDa PSA fragment. This suggests that high concentrations of a PSA degradation fragment can indeed be found in serum samples with high concentrations of the native PSA molecule. It remains to be determined whether, or to what extent, the storage conditions of a serum specimen with extreme PSA elevations can result in a PSA value for undiluted sample that unexpectedly reads below the high calibrator. However, purified PSA does not
produce a hook response at the concentrations discussed here, so we suspect that a sample stored under conditions at which it is stable will not hook at PSA concentrations of 5000–10,000 µg/L. Until this is determined it would be prudent to promptly store specimens at 4°C or to freeze them when analysis within 24 h is not possible. It is also conceivable that some other co-existing clinical condition in the patient could contribute to such an observation. For example, very little is known about the metabolism and clearance of PSA. It remains to be seen whether impaired renal clearance of PSA metabolic breakdown products could be a factor in these observations. The clinical history of these patients should therefore be carefully examined.

Bodor et al., in their Letter, and Althian and Stenman (2) have suggested using a two-step procedure to avoid any potential to observe a hook. However, the need to do so will depend on the frequency with which patients’ samples exceed 5000–10,000 µg/L. In our experience and that of several laboratories, a patient’s serum with a PSA concentration >5000 µg/L is very unusual, seen only in very advanced disease such as Stage D prostate cancer patients with scintigraphic evidence of bone metastases, and would be unlikely to create a clinical dilemma. The true frequency of extremely high values for PSA is not known with certainty and may depend on the patient population that is being seen. A conservative alternative to a two-step protocol would be to decrease the value of PSA above which the specimen should be repeated on dilution—e.g., repeat, after dilution, those samples that initially read between 50 and 100 µg/L. This will identify whether any patients’ samples that exhibit the high-dose hook effect are present in the patient population under study.

References

Bodor et al. respond:

To the Editor:

We thank Dr. Myrtle for his response regarding the high-dose hook effect in PSA measurement (1). Dr. Myrtle states that, “...three separate studies... with the use of purified PSA suggest... the Tandem-R PSA method will produce a sample response generally exceeding the high calibrator until the PSA concentration exceeds 10,000 µg/L.” However, in actual practice with patient material we and others (1–9) have demonstrated samples with PSA values of 7.5, 8.6, and 8.9 mg/L to “hook” below the highest standard. Thus, the purified material may behave differently than endogenous PSA in this assay.

To eliminate the potentially misleading nature of hook effects, Myrtle suggests “...repeat, after dilution, those samples that initially read between 50 and 100 µg/L.” Yet, we have clearly shown a “hook effect” to near the normal range (4.6 µg/L) for our patient whose value for PSA was 50.6 mg/L (1, 4). Thus, if one is to detect such rare cases, one must extend Myrtle’s recommendation to all samples, which has always been our practice. Failure to do so may relay contradictory and confusing information to the clinician after treatment of the patient.

We agree with Myrtle that a possible cause of this hook effect could be autodigestion. Autodigestion of PSA molecules may indeed explain the appearance of lower-molecular-mass PSA fragments that Althian and Stenman (2) thought caused a “hook effect in their one- and two-step assays.” However, we have never observed a hook effect in our two-step assay. Possibility, there are subtle differences in storage or assay conditions between our method and theirs. Because we have never seen a hook effect in our two-step assay (1), we still think that the two-step modification is a valid and cost-effective alternative to performing one-step assays on undiluted and diluted samples for every specimen received.

References

Geez Bodor
Bryan A. Wolf
Barbara Hinds
Moon H. Nahm
Mitchell G. Scott
Barnes Hospital
Washington Univ. School of Med.
660 S. Euclid Av.
St. Louis, MO 63110

Interference of Macro-Creatine Kinase in Determination of CK-MB in Serum with the Kodak Ektachem

To the Editor:

The “Ektachem” method for creatine kinase MB isoenzyme determination is an immunoinhibition assay based on partial inhibition of creatine kinase activity in the sample—activated by N-acetylcyesteine—by antibodies to creatine M subunit. The residual enzyme activity under these conditions is ascribed to creatine kinase MB and BB isoenzymes. Bissell et al. (1) have evaluated the Ektachem CK-MB slide and concluded that this method is convenient for tests of serum and that its accuracy compares with that of wet chemical immunoinhibition methods. We share this view, but we report here some cases of macro CK interference with this method.

Serum samples from seven patients were studied. Total serum CK activity (2) was measured at 37°C with reagents from Boehringer, Mannheim, F.R.G., in an ACP 5040 analyzer (Eppendorf). CK-MB isoenzyme activity was assessed by electrophoresis on cellulose acetate gel, with use of a system