creased CK-MB results when assayed with the original wash solution (SW-III). The results (Table 1) show that three of the patients showed normalization of CK-MB to <7 µg/L with the SW-IV solution and would not have met our criteria for re-assay with ferritin-blanking tabs. However, blank concentrations were still high. Interestingly, patient C normalized despite an initially above-normal CK-MB result, and patient D retained a significantly increased false-positive CK-MB despite use of SW-IV. Ultracentrifugation had no effect on any of the samples tested.

These data support the data of Butch et al. (2), in which several falsely increased CK-MB samples failed to normalize with use of wash solution IV. Although more time will be needed to assess the incidence of false-positive CK-MB results when the reformulated wash solution is used, this clearly remains a problem. We continue to recommend that ferritin blank tabs be run with all samples to which CK-MB concentration exceeds the upper reference range, when the Stratus CK-MB system is used for evaluating myocardial infarction.

References

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Effects of Dialysis on Intact Parathyrin in Patients with Chronic Renal Failure

To the Editor:

We were interested to read the observations of Dilena and White (Clin Chem 1989;35:1549–4) which confirm our findings in the evaluation of the INCSTAR PTH kit. We would like to point out another area of concern regarding the interpretation of serum parathyrin (PTH) measurements in chronic renal failure (CRF). Most patients with CRF have increased concentrations of PTH in serum (1). PTH may have a role in the development and progress of renal osteodystrophy (2,3), a possibility supported by the results of initial trials of vitamin-D-induced suppression of PTH in patients with CRF (4).

Although, as Dilena and White suggested, the accumulation of C-terminal fragments may interfere with measurement of intact PTH in patients with CRF, we studied the effects of the dialysis procedure itself on the concentrations of PTH measured in serum with the INCSTAR intact PTH assay (INCSTAR Corp., Stillwater, MN). Peripheral venous blood was sampled from eight unselected patients just before a dialysis session and again at the end of the session. Four patients were receiving hemodialysis treatment and the others chronic ambulatory peritoneal dialysis (CAPD). The serum was analyzed for PTH, total calcium, albumin, and creatinine (Table 1).

In contrast to CAPD, hemodialysis markedly affects serum PTH, which may decline to 30% of its pre-dialysis value in a single session. These preliminary results show that the time of sampling for PTH measurement in patients on hemodialysis must be standardized for valid comparisons to be made over time. We do not yet know whether pre-dialysis or post-dialysis samples best reflect parathyroid gland activity or the tendency to develop renal osteodystrophy.

Until these questions are settled, no firm guidelines can be offered in relation to sampling for PTH measurement in patients on hemodialysis, and published results must be interpreted with caution.

References
1. Bicknell EJ, Van't Hoff W. The use of an intact molecule parathyroid hormone assay

Table 1. Effects of Dialysis on PTH, Calcium, Albumin, and Creatinine in Serum of Eight Patients

<table>
<thead>
<tr>
<th>Patient</th>
<th>PTH, ng/L</th>
<th>Ca, mmol/L</th>
<th>Alb, g/L</th>
<th>Cret, µmol/L</th>
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*Numbers in parentheses: reference interval for healthy patients. 2 The first value in each set is the pre-dialysis value; the second is the post-dialysis value.


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Possible Overestimation of Ultracentrifugally Isolated "Free" Apoprotein A-I in Serum

To the Editor:

We read with great interest the report by Duval et al. (1) describing the concentration of free apolipoprotein (apo) A-I in serum of patients with end-stage renal failure. They cited one of our studies (2) but, surprisingly, with an erroneous interpretation. Our patients showed normal serum apo A-I (or rather, not significantly different from controls) and a significantly greater ratio of apo A-I to HDL cholesterol than in the controls (2). Similar results have been obtained in a study of patients with chronic renal failure who are undergoing hemodialysis in the Seattle area (3). We wonder if such disagreement could be due to some of the characteristics of the control group studied by Duval et al. (1), these being significantly younger than the uremic patients and with most of the women taking oral contraceptives.

Some authors (4, 5) have demonstrated that ultracentrifugation has a deleterious effect on apo A-I-containing lipoproteins as well as on other particles normally bound to lipoproteins. If this is true, then ultracentrifugation doesn't seem suitable for separating apolipoproteins apparently not bound to lipoproteins. A note of caution should be added to interpretation of results so obtained, at least to the quantitative aspects.

However, preliminary results of a project we are conducting in Seattle confirm some of the results reported by Duval et al. In plasma from uremic patients, particles containing A-I and A-II as well as particles containing A-I but not A-II, isolated by the method described by Cheung and Albers (6), showed a striking heterogeneity when compared with those in plasma from controls. Some of the particles found have an extremely low Stokes diameter; probably these account for the increased concentration of "free" apo A-I in serum. These small subpopulations were present irrespective of the concentration of hepatic lipase activity and the presence or not of triglyceride-rich lipoprotein remnants—evidence that is apparently against a substantial role for these particles in the metabolism of these lipoproteins. We also confirm the absence of immunoactive apo A-I in plasma ultrafiltrates from these patients.

Finally, this comment on another point in the field of terminology: Unfortunately, "freedom" is a rare and ever-changing concept that deserves a more philosophical approach. In the case of a protein that is extraordinarily avid for lipids and is present in our biological "milieu," freedom is only a utopian possibility. In the absence of a better expression, the adjective "free" should be written with quotation marks.

References

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Stability of AST and ALT Assays in Tris Buffers

To the Editor:

Wroblewski and LaDue (1) described the first kinetic assay for alanine aminotransferase (ALT, EC 2.6.1.2) in serum. Analyses for ALT and aspartate aminotransferase (AST, EC 2.6.1.1) are now done by the methods recommended by the International Federation of Clinical Chemistry (IFCC) (2–4). The principle of the methods is the same but there have been the following changes: (a) substrate concentrations were optimized; (b) Tris buffers are used instead of phosphate; (c) combined buffer and serum are pre-incubated to allow reactions with NADH; and (d) the reaction is measured after the substrate, alphaketoglutarate, is added.

Our clinical research requires us to re-analyze some patients' sera, sometimes after several months during which the specimens have been stored at −20 °C. To compare the performance of the IFCC reagents in this assay under these conditions, we analyzed aliquots of 50 different patients' serum specimens (Set 1) for ALT and AST before and after freezing at −20 °C. A Hitachi 736 analyzer and the BMD-system packs (cat. nos. 797359 and 798029; Boehringer Mannheim Diagnostics, Indianapolis, IN) were used for the analysis. Freezing did not alter the AST activity, but there was substantial loss of activity of ALT.

Tris buffers have been shown to inhibit recombination of the apoenzyme with the cofactor, pyridoxal 5-phosphate (P-5-P). We therefore assayed the frozen specimens after activation with this cofactor, which partly restores the original activity. Because the availability of reagents for the older (pre-IFCC) method was limited, we analyzed fewer (20) patients' sera samples (Set 2) with these reagents before and after freezing at −20 °C. Freezing did not affect measurements of AST or ALT activity when we used these reagents.

To determine whether this loss in activity is related to freezing and subsequent thawing or simply takes place slowly with time, we analyzed 15 serum samples (Set 3) that had been stored at 4 °C for three weeks, performing the ALT and AST analyses after two weeks of storage and at the end of the three weeks. The results in Table 1 support the relationship of the freeze–thaw process to the loss of ALT activity.