very high concentrations of ACTH. There was no difference in mean decay time in samples of controls and patients.

We found a significant change in ACTH plasma concentrations with time, as in other studies (1, 5), but this change was much smaller than expected. Unlike another investigation (5), we studied samples not only from healthy volunteers but also from patients with high ACTH concentrations. Our study did not show a difference in the mean rate of hormone concentration change in high-ACTH samples vs normal samples. In our study, for up to 24 h the decline in the measured ACTH concentration was \( \pm 10\% \) even in whole blood stored at room temperature. Given the analytical imprecision of \( \pm 15\% \), commonly accepted for immunoassays, a 10% change in the hormone concentration attributable to preanalytical factors seems not to be a major problem in a clinical setting. We therefore confirm stability of ACTH in EDTA plasma for \( \pm 24\) h as previously reported (5) for a manual radioactive version from the same assay from the same manufacturer. Sample temperature during the preanalytical phase appears to have less influence on measured ACTH concentrations than does time to centrifugation. We speculate that enzymes involved in EDTA degradation are not inhibited sufficiently at 4 °C.

Although the mean decay in measured ACTH concentration after storage for 24 h at room temperature without centrifugation was only 10% [mean (SD), 9% (11%)], the decrease was \( >20\% \) in samples from 3 healthy volunteers and was not prevented by storage at 4 °C. No relevant change occurred in any of the samples during the first 4 h, however. For clinical practice we therefore recommend that centrifugation and separation of plasma supernatant be performed within 4 h of sample collection. Cooling of samples seems to be much less effective. Thus, the preanalytical procedure can be simplified without risking clinically relevant changes in measured hormone concentrations.

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Indican Interference in Bilirubin Assays: A Classical Solution Still Applies

To the Editor:

Abbott Laboratories recently supplied a new reagent for total bilirubin (catalog no. 61.45-20) for use with the Architect cSystems analyzer. The reagent uses 2,4-dichlorophenyl diazonium (2,4-DCPD) and is described as minimizing interference from hemoglobin, although interference from indican was reported to be higher than with the previous reagent (1). Our initial comparison of the new and previous reagents yielded a regression equation with a slope of 1.05 (Fig. 1, upper panel, ■) and similar imprecision (not shown) for the 2 reagents. After introduction of the new reagent into routine use, however, bilirubin results in renal dialysis patients were noted to be higher than with the previous reagent. Among –512 predialysis samples received during a 3-day period from patients on renal dialysis, 43% had bilirubin values above the upper limit of the reference interval (3–13 mg/L). For most of these patients, bilirubin concentrations reported within the previous 1–2 months had been within the reference interval.

We measured bilirubin with both the old and new reagents in a group of predialysis renal patient plasma specimens (Fig. 1, upper panel, □). The slope of the Deming regression equation was significantly higher in the renal group than it was in the initial method-comparison study using unselected leftover laboratory specimens (1.33 vs 1.05; unpaired t-test, \( P < 0.0001 \)). Interestingly, with the new reagent the absorbance continued to increase after the first minute in renal dialysis samples, but not in nonrenal samples (Fig. 1, middle panel).

The findings suggested interference from indican, a metabolite that increases in uremia (2). After addition of indican, total bilirubin results with the 2,4-DCPD and 2,5-DCPD methods were reported to increase by 50 and 33 mg/L per mmol/L of indican, respectively (2). Abbott reported (1) that, with the new reagent, the bilirubin increased by 15 mg/L for 0.25 mmol/L of added indican as compared to a 17 mg/L increase for 0.50 mmol/L of indican using the old reagent. Indican concentrations up to 0.38 mmol/L have been observed in predialysis serum samples from renal failure patients (3).

To test the effect of indican, we added indican (indoxyl sulfate; Sigma-Aldrich) to a plasma pool generated from patients with normal renal function. The time course of absorbance for the new Abbott bilirubin assay matches that seen during analysis of plasma from dialysis patients. In the absence of added indican,
near-maximum absorbance was achieved with the new reagent at ~0.9 min (Fig. 1, lower panel) and remained relatively constant through the rest of the 5.1-min period during which the analyzer monitored the reaction. In the presence of 0.4 mmol/L of added indican, the absorbance was higher at 1 min and continued to increase for at least 5 min (Fig. 1, lower panel).

As suggested by McPhaul et al. (4) in 1985, indican interference may be markedly reduced by the use of an early reading time of 1.7 min (4). As shown in Fig. 1 (middle panel), we expected that the influence of indican would be decreased by ~75% if we used a reading time of 1.2–2.4 min rather than the manufacturer’s recommended time of 4.2–5.1 min (Fig. 1, middle panel). The predialysis specimens from 223 renal dialysis patients with abnormally high bilirubin values, as measured with the new reagent (reading time, 4.2–5.1 min), were reanalyzed using both the early (1.2–2.4 min) and late (4.2–5.1 min) reading times for the new reagent. Total bilirubin values decreased by a mean (SD) of 9.0 (2.0) mg/L with the early reading time, and 97% of these values were then within the reference interval. Use of the earlier reading time did not affect day-to-day imprecision (CV, 2.9% and 2.7% at 61 mg/L for late and early reading times; n = 36).

The problem of indican interference in 2,4-DCPD and 2,5-DCPD methods for total bilirubin measurement, reported more than 30 years ago (2, 3), continues to be problematic. This report is intended to remind the laboratory community to evaluate the effect of indican in new formulations of these reagents. Fortunately, the simple solution proposed by McPhaul et al. (4) more than 2 decades ago still appears to be effective for decreasing indican interference in current bilirubin assays.

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Fig. 1. Effect of indican interference on a new bilirubin reagent formulation.
Upper panel, comparison of results of previous and new methods for routine (n = 32; closed squares) and renal (predialysis) patient plasma samples (n = 29; open squares). The Deming regression equation for the routine samples was as follows: Bilirubin_{new reagent} = 1.05 \times \text{Bilirubin}_{previous reagent} + 2.0 mg/L; r = 0.87; S_{xy} = 3.0 mg/L; slope 95% confidence interval (CI; 0.82–1.28). For the renal specimens it was as follows: Bilirubin_{new reagent} = 1.33 \times \text{Bilirubin}_{previous reagent} + 1.9 mg/L; r = 0.92; S_{xy} = 1.9 mg/L; slope 95% CI (1.10–1.56). Middle panel, absorbance (548 nm) vs time for a nonrenal patient sample (closed squares; total bilirubin = 27 mg/L) and renal dialysis patient sample (open squares; total bilirubin = 20 mg/L) using the new reagent. Lower panel, absorbance (548 nm) vs time in the absence (closed squares) or presence (open squares) of added indoxyl sulfate (0.4 mmol/L) with the new reagent formulation. The bilirubin concentration of the pooled patient plasma was 4 mg/L.
LDL Particles Are Nonspherical: Consequences for Size Determination and Phenotypic Classification

To the Editor:

The recent article by Ensign et al. (1), reporting on a disappointingly low agreement among 4 methods to assess LDL particle size and phenotypic classification, casts doubt on the utility of these techniques in clinical practice. A similar poor correlation ($r = 0.39$) between LDL size measurements by nuclear magnetic resonance (NMR) and gradient gel electrophoresis (GGE) has been reported by Witte et al. in a study with 324 individuals (2). Ensign et al. (1) advocate a standardization program to reduce the lack of concordance between methods. In addition to standardization problems, however, there is another reason for the observed discrepancy that is not addressed in the Ensign paper and accompanying editorial; the assumption that LDL size is adequately described by a single variable, diameter.

We would like to point out that this assumption may not be valid for LDL particle size. The situation is akin to the quantification of obesity, for which several measures are commonly used, such as body mass index, waist circumference, and waist-to-hip ratio. Although these measures are significantly correlated, they cannot be used interchangeably. If humans were spherical objects, the agreement between these measures of obesity would be perfect. But this is obviously not the case, because human beings come in all sorts of shapes. We think that to a certain extent this is also true for LDL particles. Although a spherical shape may seem intuitively right, several experimental approaches have not confirmed this characteristic of LDL particles but instead suggest that they are nonspherical. Nonspherical shape is not unique to LDL particles, but also occurs within the HDL class of lipoproteins. Although mature HDL particles are spherical, it is well accepted that nascent HDL particles are discoidal.

The fact that each LDL particle contains a single copy of apolipoprotein B-100, almost fully accounting for the protein content of LDL, allows straightforward calculation of average LDL particle volume from its chemical composition (3). Assuming a spherical particle shape, average diameter can then be calculated by simple arithmetic. In a study including 160 individuals, we observed that LDL diameters measured by high-performance gel-filtration chromatography correlated poorly ($r = 0.60$) with calculated diameters (3). This discrepancy could be reconciled by assuming that LDL particles are discoidal, with a mean diameter of 20.9 nm (range 19.6–21.6 nm) and a mean height of 12.1 nm (range 10.5–13.9 nm) (3). These values are in striking agreement with dimensions obtained by cryo-electron microscopy, which is a technique allowing visualization of single LDL particles from different angles (4). Furthermore, data obtained by crystallographic analysis are also indicative of a pseudocylindrical or discoidal particle shape (5).

An important consequence of the discoidal LDL model is that techniques that are currently used to assess LDL size are not equivalent. Techniques such as dynamic light scattering and NMR actually measure particle volume, from which diameter is calculated, assuming a spherical particle shape. This principle also applies to density gradient ultracentrifugation, because density is inversely proportional to particle volume. In contrast, measurements by high-performance gel-filtration chromatography and electrophoretic techniques such as GGE and tube gel electrophoresis probably reflect particle diameter more closely. A striking feature of the discoidal particle model is that diameter and height are not significantly correlated (3).

Consequently, a flat particle of large diameter can have the same volume as a thick particle of small diameter, resulting in similar NMR readings but widely differing GGE results.

In conclusion, we suggest that the lack of agreement between various methods to assess LDL particle size or phenotype is partly due to the fact that LDL particles are not spherical and therefore their size cannot be described by a single variable. In addition to partly resolving the perceived discrepancy between LDL size measurements, the discoidal particle concept puts a new perspective on the notion that small, dense LDL are more atherogenic than their large counterparts. Clinically, discoidal particle shape raises the question of what measure of LDL size or shape—volume, diameter, height, or aspect ratio—is most closely related to cardiovascular disease, an evaluation process reminiscent of the ongoing discussion of whether body mass index or waist circumference is a better predictor of cardiovascular outcome. Unfortunately, in contrast to these anthropometric measures, which are readily performed on large numbers of individuals, measurement of LDL dimensions is not easily performed on a large scale. Nevertheless, we do think that it would shed more light on the relative atherogenicity of specific LDL subclasses.

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