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Dialysis and Ultrafiltration Give Different Measures of Binding of Divalent Cations to Serum Proteins

To the Editor:

It has been suggested that the fraction of calcium bound to proteins includes a more tightly bound portion (1) that might be detected only at very low concentrations. To study the binding of calcium and magnesium to human serum proteins at infraphysiological and normal concentrations, we determined the percentages of each element bound, using two techniques: dialysis according to Ladenson and Shyong (2) and ultrafiltration according to Farese (3); concentrations were measured by atomic absorption spectroscopy. The infraphysiological concentrations were obtained by use of cation-exchange chromatography (4).

Figure 1 illustrates the different results obtained with the two techniques. The calcium binding percentages at physiological concentrations obtained by ultrafiltration were 12% (SD 2.4%) higher than those obtained by dialysis, and the magnesium ultrafiltration values were 9% (SD 1.9%) higher than those by dialysis. The results found with ultrafiltration are more consistent with those reported by other workers (5). Besides, with the dialysis technique we saw an increase in the percentages bound at calcium concentrations lower than 0.4 mmol/L and at magnesium concentrations lower than 0.1 mmol/L. These increases were not found with the ultrafiltration techniques.

These dialysis findings are consistent with there being a fraction that is tightly bound to proteins, as reported by Worstman and Tracoff (1), but our ultrafiltration results do not support this conclusion. Rather, as was pointed out by Keresztes-Nagy et al. (6), the values obtained at low concentrations may be influenced by adsorption of ligand to the bag, which may indeed be the explanation for this fraction that supposedly is tightly bound to proteins.

References

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Improved Detection of Chylomicrons in Serum

To the Editor:

Two procedures are commonly used to detect chylomicrons in human plasma, the standing plasma test and lipoprotein electrophoresis. The 16-h-standing plasma test fails to detect chylomicrons in about 80% of cases, but in these cases they can be detected by electrophoresis (1). However, lipoprotein electrophoresis is expensive and time consuming.

Chapman has proposed a rapid procedure (2) for detection of chylomicrons by centrifugation.

After centrifugation of plasma in Natelson capillary tubes, chylomicrons, if present, range from a prominent creamy layer to one visible only by using a 10× lens against a dark background. We confirmed the sensitivity of this procedure as compared with lipoprotein electrophoresis. However, the poor standardization in the detection of the creamy layer and possible mistakes in samples with fibrin debris make this procedure rather inaccurate, with a consistent proportion of false positives—which are even more frequent in the plasma of hemodialysis patients.

We improved Chapman's method by using serum instead of plasma, centrifuging the samples for 10 min in a standard microhematocrit centrifuge. We mix a 100-μL aliquot of the serum with 10 μL of a 2.5 g/L solution of Nile Blue sulfate and fill capillary tubes with 80 μL of this serum. For detection of chylomicrons at the top of capillary tubes we use a microscope (objective 2.5×, oculars 10×).

Chylomicrons are clearly detectable in samples from nonfasting persons, appearing at the top of the capillary tubes as a red or red-orange layer. Neutral lipids (triglycerides) extract from the aqueous solution of Nile Blue only the oxazine and free base, both red in color. Hydrolyzed solutions of Nile Blue therefore consist partly of the blue salt and partly of the red free base and the red oxazine. During centrifugation only chylomicrons float; thus the test is highly specific.

The microscopic pattern of chylomicrons was similar in cases of endogenous chylomicronemia (type V hyperlipoproteinemia).

We compared the sensitivity of this new procedure with that of lipoprotein electrophoresis on agarose ("Bio-Gram A," Bio-Rad Laboratories) in five cases of postprandial chylomicronemia at different times after the meal, or by diluting the samples with a pool of normal sera. The limit for the detection of chylomicrons was twice as efficient with our microprocedure.
The specificity was studied by comparing results obtained with the proposed procedure with those obtained with lipoprotein electrophoresis for 80 normolipidemic or hypertriglyceridemic (>4.0 g/L) samples. A reasonable percentage of false negatives was detected in hypertriglyceridemic sera with lipoprotein electrophoresis, owing to the lesser sensitivity of this procedure.

We conclude that our proposed modification of Chapman’s procedure (2) offers the following advantages: (a) serum can be used instead of plasma (in general, serum is more widely used in clinical chemistry laboratories); (b) the instrumentation (capillary tubes and hematocrit microcentrifuges) is available in all such laboratories; (c) microscopic detection of chylomicrons is easier and more objective; (d) no false positives were demonstrated in samples with fibrin debris or other “artificial” particles; and (e) the microprocedure appears to be more sensitive than lipoprotein electrophoresis.

References

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Photographic Monitoring of Enhanced Luminescent Immunoassays

To the Editor:

We report a new procedure for performing luminescent immunoassays, in which instant photographic film is used to detect the light emission. It exploits the finding that the relatively high and constant light emission from the firefly-luciferin-enhanced chemiluminescent reaction of luminol peroxide with horseradish peroxidase conjugates (1) can be detected by high-speed instant photographic film. Advantages of photographic detection are that it is rapid, many samples can be assayed simultaneously, a permanent visual record of the results is obtained, and the equipment is compact and uncomplicated, requiring no external power source. We illustrate the applicability of this procedure by results of an immunoassay designed to screen sera for low concentrations of ferritin.

Photographic film has been used as a detector in a range of luminescent assays, e.g., for metal ions (2), ozone (3), glucose (4), enzymes (5), and luminol derivatives (6), the results being assessed either visually or quantified by densitometry, spot size, or by using an optical step wedge. Until now, photographic monitoring of luminescent immunoassays has not been possible because the rapid emission of light necessitated initiation of the luminescent reaction in front of the film, and the low light intensities were not readily detected, even with highly sensitive film.

In contrast, emission from the recently discovered luciferin-enhanced chemiluminescent reaction of luminol peroxide with horseradish peroxidase conjugates is high enough that photographic film can be used as a detector. Also, the light emission is constant over a period of minutes and thus luminescent reactions can be initiated before exposure to film.

Many immunoassays are done in microtitre plates, but in the past luminescent immunoassays in which such supports were used required complex photomultiplier-based plate-readers with an integral injection system for luminescent assay reagents (7,8). Instant film, however, provides a simple, inexpensive alternative, with which one can simultaneously monitor as many as 60 wells of a microtitre plate. The equipment required is a light-tight box containing the film, a shield to house the microtitre plate and isolate light emission from individual wells, a simple shutter to control the exposure time, and a conventional multiple pipette. A similar approach has been used in radioimmunoassay, based on an aluminum shield and an X-ray film (9), but it requires overnight exposure, whereas the luminescent method described here requires only a 30- to 60-s exposure time and 35 s of development time.

Figure 1 illustrates results obtained when Polaroid Land instant film was used to monitor a luciferin-enhanced luminescent enzyme immunometric assay of a series of ferritin standards. A graded response is obtained, which readily allows visual discrimination between the ferritin standards. The application of this assay to a series of patients’ samples is shown in Figure 2.

The response of the film is such that it is completely exposed by samples with ferritin concentrations exceeding 20 ng/mL. Below this, the degree of exposure is intermediate, so that samples from patients with abnormally low ferritin concentrations can be immediately identified.

Assay conditions can be adjusted so that the film is exposed only above a certain concentration of analyte, thus making it ideal for screening purposes. It is not restricted to microtitre plates; other solid supports such as tubes, microencapsules, beads, or dipsticks can also be used in conjunction with the film. Further improvements in the degree of enhancement of light emission and reduction in incubation times with sample and conjugate will extend its applicability.

This technique lends itself to many of the immunoassays involving horseradish peroxidase conjugates, particularly those used for screening for (e.g.) hybridomas or specific antibodies, or in meat-species testing or allergy panels. In a preliminary study it was possible to screen sera for antibody against cytomegalovirus with a 1-h assay, thus further illustrating the feasibility of photographically monitored luminescent immunoassays.

Fig. 1. Solid-phase firefly luciferin-enhanced luminescent enzyme immunometric assay of a series of ferritin standards (0–20 ng/mL) monitored by use of instant photographic film. Shown is part of a polyvinyl chloride microtitre plate (Dynatech M24) coated with rabbit anti-human ferritin antibody which was incubated with serum samples or standard (100 μL) for 2 h and then with horseradish peroxidase-labelled rabbit anti-human ferritin antibody (100 μL) for 3 h at room temperature. After the plate had been washed, we determined residual peroxidase activity in the wells by adding 200 μL of an assay mixture containing, per liter, 1.25 mmol of luminol, 39 μmol of firefly luciferin, and 2.7 mmol of hydrogen peroxide, in Tris HCl buffer (0.1 mol/L, pH 8.0) with the aid of a Tilttek multichannel pipette. The glowing microtitre plate was placed in the shield on top of the Polaroid Land instant film (20 000 ASA, Type 612) and the film was exposed for 30 s, then developed for 35 s.

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