Whole Plasma or Plasma Water: the Problem of the Reference Base

To the Editor:

It is our responsibility to decide which measurements are valuable in the investigation of the basic pathology of disease and, after discussion with clinicians, in patient care. It is then our responsibility to discuss with instrument manufacturers how these measurements can be made, and industry then takes over the responsibility of providing suitable apparatus. Sometimes this process goes the wrong way round. A manufacturer designs a machine for measuring c.a.f. rhubarb, and we then provide these unneeded results for the clinicians.

The most recent correspondence (1, 2) on the relative relevance and validity of "whole-plasma sodium concentration" by flame photometry and of "plasma-water sodium ion activity" by electrode illustrates this point. It is up to us to discuss, and if possible to decide, which analysis is more relevant to our needs. The controversy applies to all substances measured in body fluids, not just to plasma sodium. We measure concentrations usually in (milli)moles of substance (numerator) per litre of material (denominator), and put in immense effort with reference methods to have the numerator correct to four significant figures, without really paying attention to the nature of the denominator, or reference base.

In the earlier days of our profession (3) plasma did not play the overwhelming predominant role as an analyzed material that it does now (4). We go on measuring concentrations of substances in whole plasma because it is easier, and customary. We should not be thinking of the results of plasma analyses just as static magic numbers, but in relation to rates of transfer of substances through extracellular fluid into and out of cells or glomerular filtrate, or to effects on cell membranes, or on other physiological processes. But soluble analytes are dissolved in the water compartment of plasma, and, when, for example, they are transported into cells they move in the first instance into the intracellular water. The rates of all these processes vary with the concentrations in plasma water. We are also all familiar with apparent, but pseudo-, changes in whole plasma concentration of an analyte due to changes in the proportion of water in plasma that result from changes in plasma proteins or lipids. Concentrations in whole plasma are at least one remove from the measurement that has physiological or pathological meaning.

I think we should decide that it is activity of analyte per unit of plasma water, not concentration per unit of whole plasma, that really matters. True activity is not always easy to measure, but a first approximation would be to express concentrations per unit of plasma water. This would require the manufacturers of plasma-crunchers to design and build-in a module that measures the water concentration in all plasma samples submitted, and utilizes these values in calculating the final results. We will all then need to recalculate our reference values.

References


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Extraction of Valproate from Serum—A Reply

To the Editor:

I would like to reply to some of the comments made by Goudie et al. (Clin. Chem. 26: 1929, 1980) concerning our valproate extraction method (Clin. Chem. 26: 674, 1980).

In our hands, emulsions do not form if samples are mixed by agitation two or three times as described in our Letter. However, emulsions frequently form if samples were vortex-mixed briskly.

We have had no problems with blocked needles. However, it is clear that the needle will become blocked if any of the protein-containing aqueous phase is
inadvertently allowed to dry within or on the tip of the needle.

We have been using chloroform as extracting solvent for over a year, but have not seen the problems encountered by Goudie et al. concerning detector contamination, sensitivity, and baseline noise. The baseline noise they referred to in our chromatogram is not related to the use of chloroform. The degree of noise is common to the other functioning flame detectors in our instrument, even though they have not been exposed to halogenated hydrocarbons.

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Automated Solid-Phase Assay for Estimation of Thyroxine Binding Capacity in a Screening Program for Congenital Hypothyroidism

To the Editor:

We recently described a method for evaluating the binding capacity of thyroxine-binding globulin (TBG) by use of samples spotted on filter paper in a mass-screening program for congenital hypothyroidism (1). To automate our screening program fully, we have now adapted our method to the “Systems Concept 4” (Micromedic Systems, Horaham, PA 19044), using their Neonatal T4 Kit, a system that we have been using for thyroxine (T4) measurement for the last two years.

Patience in spotting a low thyroxine on filter paper but a normal thyrotrphin concentration, two 6-mm spots on filter paper are placed in 800 μL of barbitral buffer (pH 8.6, 0.75 mol/L) in tubes coated with anti-T4 and allowed to elute overnight. The omission of normal rabbit serum and 8-anilino-1-naphthalene sulfonate from the buffer allows competition for the T4 molecule between proteins eluted from the spots and the anti-T4 antibody. The next day, radiolabeled T4 (~25 000 cpm in 200 μL of barbitral buffer) is added to the tube and the mixture is incubated for 30 min at 37 °C, as for the regular assays. The contents of the tubes are emptied out, the tubes are gently rinsed with distilled water, and the radioactivity in the empty tube is counted. Results are expressed as percent binding of the 125I-T4 to the coated tube, the “100%” tubes (quadruplicate) being tubes without eluate. In contrast to our previously reported method involving charcoal absorption (1), samples containing low concentrations of TBG will yield high values.

The within-assay CVs for normal and high values were respectively 6.0 and 6.7%. On the other hand, the between-assay CVs were 6.5% for normal values and 6.7% for high values. The mean binding for our normal population (n = 186) was established at 18.3% (SD 3.5%), compared with the low TBG (n = 20) binding of 40.3% (SD 3.4%). The correlation (r) between values obtained by this method and our previously reported method was 0.73 for 64 samples, including eight samples from patients with low TBG. Furthermore, with a cut-off point for recall established at 28%, the false-positive rate of recall is only 1%.

Reference


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Iodination of Antiferritin by the Method of Bolton and Hunter

To the Editor:

In 1977, I reported having used the Bolton and Hunter technique (1) for labeling purified antibody with 125I-labeled hydroxyphenyl propionic acid N-hydroxysuccinimide ester, for use in the immunoradiometric assay for serum ferritin (2). The conditions for the iodination procedure, the uptake of 125I, and the stability of the labeled preparation which I observed with the method were cited in this article.

Recently, Alvarez-Hernández and Loriá (3) reported the use of the Bolton-Hunter reagent for labeling antiferritin, together with the claim that theirs is the first report of the method for this purpose. They failed to include any of the conditions of the iodination, although their observed uptake of 125I and stability of the iodinated antibody were similar to that which I reported.

The significance of my earlier report, which these authors overlooked, was not in the labeling of antiferritin specifically for use in a single immunooassay, but in the application of a new technique for iodinating large proteins. The advantages offered by the method are simplicity and mild conditions which eliminate oxidation and reduction reactions in the presence of the protein to be labeled. Since my report, the method has been applied to the iodination of ferritin, a protein larger than IgG antibody, with subsequent improved storage characteristics as compared with ferritin labeled with oxidation methods (4, 5).

References


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The authors of the communication in question respond:

To the Editor:

The omission of the paper by Gonyea is the outcome of a perhaps growing problem in computer technology, as a five-year retro-search under several words or either label, antibody, antibodies, immunoglobulins, and IgG coupled to either antiferritin or Bolton and Hunter reagent) failed to detect her publication. The computer was linked to the BIOSIS system in the U.S. Our apologies to Dr. Gonyea for this inadvertent and unfortunate omission.

In regard to her questions on the iodination procedure: 50 μL of a 50% suspension of Sepharose 4B coupled to human ferritin is incubated overnight with 500 μL of rabbit serum containing antiferritin, to yield an immunoadsorbent with 50–90 μg of adsorbed antiferritin. The immunoadsorbent is incubated overnight with 500 μCi of air-dried Bolton/Hunter reagent. It is washed three times with borate buffer, and incubated for 30 min with 2 mL of 3 mol/L KSCN containing 50 g of bovine serum albumin per liter. The supernate is dialyzed against borate buffer. The final preparation is aliquoted and stored at ~20 °C.

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