Antiferritin Labeled with the Bolton–Hunter Reagent

To the Editor:

The Bolton & Hunter reagent for protein iodination (1) has been used successfully to label the ligand (ferritin) in a radioimmunoassay of serum ferritin (2) but has not been tried in the labeling of antiferritin in immunoradiometric assays. We have labeled antiferritin with the Bolton & Hunter reagent (iodinated p-hydroxyphenylpropionic acid N-hydroxysuccinimide ester), and used it in the two-site immunoradiometric assay developed by Miles et al. (3). In addition, inclusion of an extra step in the assay procedure has improved the sensitivity and the linearity of the standard curves.

We have used the assay procedure described by Miles et al. (3) with slight modifications in volume and with an extra step included: after the polystyrene tubes are coated overnight with 200 μL of cold antiferritin and washed, they are again incubated overnight with 400 μL of isotonic saline–phosphate buffer (0.1 mol/L, pH 7.4) containing 5 g of bovine serum albumin per liter, and washed before proceeding with the successive coating with standards (or unknowns) and labeled antiferritin.

Our seven attempts to iodinate the antiferritin with the Bolton & Hunter reagent have all been successful. Iodine uptake has ranged from 25 to 35% of added radioiodine, and the infinite dose response in the assay (up to 1000 μg of ferritin per liter) has been reproducible in the seven iodinations, ranging from 46 to 56% of the labeled antiferritin added. The stability of the labeled antiferritins has been evaluated by the changes in the infinite dose response in the course of time. Figure 1 shows the findings in three of our preparations which show some differences in stability, i.e., T1/2 of about 40 days for preparation B and about 80 and 100 days for preparations A and C, respectively. The reasons for these differences are unknown to us but all three preparations gave adequate standard curves.

The inclusion of an extra incubation step with buffered bovine serum albumin (either 5, 10, or 20 g/L) usually increases the slope of the standard curves and tends to linearize the concentration/cpm curve over the tested range of 0 to 25 μg of ferritin standard per liter (correlation coefficients of 0.990 to 0.999 in 20 batches in which a straight line was calculated by the least-squares method). The latter finding contrasts with the logit-log response seen by Miles et al. (3), and with the nonlinear response seen by us when the bovine serum albumin concentration is decreased to 1 g/L. The effects observed do not appear to be the result of a decrease in nonspecific binding, because the zero dose counts are very similar with and without the extra step. We think it may be due to a “reorientation” of the cold antiferritin (which would then bind ferritin more easily) brought about by the presence of albumin molecules sticking to the tube walls in between the antiferritin molecules.

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References

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Radioimmunoassay of Cortisol in Blood Collected on Filter Paper

To the Editor:

Heel-prick blood samples, collected on filter paper, are widely used nowadays for neonatal screening of phenylketonuria (1). Radioimmunoassay of thyroxine and thyrotropin in blood specimens on filter paper has also been shown to be a practical means for screening newborn infants for hypothyroidism (2, 3). Pang et al. (4) first applied the filter paper method for measuring a steroid, 17α-hydroxyprogesterone. The advantages in terms of sample size, handling, and transport provide a potent stimulus for broader applications of the method. The relatively high concentrations of cortisol in blood, as well as its clinical importance, make this steroid hormone a suitable candidate.

Capillary blood obtained by finger-prick from normal adults was applied as 1-cm spots onto filter paper (Schleicher & Schuell, no. 903) identical to that used in screening for phenylketonuria and hypothyroidism. Venous blood specimens were simultaneously obtained. A paper disc, 6.4 mm in diameter, was punched out from the central region (5) of the air-dried blood spots with a hole puncher. 125I] serum cortisol radioimmunoassay kits, kindly donated by Clinical Assays, Cambride, MA 02139, were used to estimate cortisol in the paper disc and in the corresponding venous serum. The procedure involves the following steps: addition of phosphate-buffered isotonic saline + [125I]-labeled cortisol and serum (or standard) to antibody-coated tubes, and 45-min incubation at 37 °C, followed by decantation and counting. It was modified as follows to measure cortisol in the paper discs. Buffer and tracer were added to the antibody-coated tubes containing the paper disc, including the tubes with cortisol standards (0, 100, 300, 1000, 2500, and 6000 pg) to which a blank non-apotoped paper disc was