Total bilirubin in the cord serum was 14 mg/L, which correlated with the delta 450 bilirubin estimate of 15.2 mg/L. The free hemoglobin in the cord blood was 68 mg/L. The cord blood hematocrit was estimated to be less than 5%.

As shown by the data listed in Table 1, the mother had demonstrated an increasing anti-D titer throughout the pregnancy (highly suggestive of Rh immunization); however, the amniotic fluid concentrations of bilirubin were low and therefore inconsistent with the anti-D titers. The amniotic fluid did not show visible signs of hemolysis or methemoglobin in any specimen. In fact, decreasing concentrations of bilirubin were observed at weeks 30, 31, and 32, and by week 35 the bilirubin estimation by the delta 450 test was quite low (estimated value of 0.4 mg/L).

The low result for bilirubin, and hence the failure of the delta 450 test to reflect accurately the apparent intra-uterine hemolysis, was presumably due to the low hematocrit. We conclude that because of the low mass of erythrocytes in the fetus, there was insufficient hemolysis to show an increased bilirubin concentration in the amniotic fluid.

The reviewers of this Letter have suggested that another useful test in this situation would have been the determination of the IgG subclasses in the maternal blood.

References

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Microbiological and Radioimmunological Assays for Folic Acid In Whole Blood Compared: Effect of Zinc Nutriture

To the Editor:

Radioimmunoassays (RIAs) for folic acid in the blood, for which several kits are available, offer advantages of speed and technical ease over the microbiological assay. However, microbiological assay for folate in blood is still considered to be the most sensitive and reliable method. Lactobacillus casei, the organism long used for folate bioassay, responds similarly to all physiological derivatives of the pteridine moiety of the vitamin, a feature not true of most radioassays (1).

Several investigators have compared microbiological and RIA methods of analysis for folate in serum or whole blood (2–5). In most instances, the two methods were comparable with large numbers of samples, but unexplained discrepancies of individual samples have been noted (2, 3, 5). During a study of mild zinc deficiency in men we observed an apparent zinc effect on folic acid metabolism as reflected in the comparison of microbiological and radioimmunoassay of folic acid in whole blood.

Three men, ages 19 to 27, were fed diets on a three-day menu rotation, consisting of conventional foods chosen to minimize zinc content and variability of composition. These diets contained approximately 150 µg of folate and 3.5 mg of zinc per day (by analysis). One man’s diet was supplemented with 400 µg of folic acid every other day. Zinc was supplemented according to the following protocol: 31 days of control, low zinc diet supplemented with 4 mg of zinc daily; 120 days of depletion, low zinc diet; 24 days of replenishment, low zinc diet supplemented with 30 mg of zinc daily. The men’s body weights were kept constant by adjusting energy intakes and expenditures. Details of these studies are published elsewhere (6).

Two folate assay methods were compared. We determined serum and erythrocyte folate activities by a microbiological procedure (7), using a chloramphenicol-resistant strain of L. casei, and also by a Becton Dickinson folate radioassay kit.1 Erythrocyte folate was then calculated by using values for whole blood, serum, and hematocrit. As the tabulation shows, dietary zinc influenced the comparative results of the two procedures.

The two methods appeared comparable during the initial control period. During zinc depletion, however, significant differences between the two folate methods were seen in both the folate-supplemented and unsupplemented groups, the apparent RIA folate values being significantly lower than those measured microbiologically. Microbiological values for erythrocyte folate concentrations remained essentially constant in the men who did not receive the folate supplement, regardless of the zinc content of the diet. On the other hand, apparent folate concentration as measured by the RIA procedure significantly declined when the men were receiving 3.5 mg of zinc per day. When dietary zinc was supplemented to 33.5 mg per day, the apparent erythrocyte folate increased slightly. When the diet was supplemented with folic acid, microbiologically measured erythrocyte folate concentration increased with time throughout the study, whereas erythrocyte folate measured by RIA did not change. No significant changes in plasma zinc were observed. Addition of exogenous zinc to pooled specimens of plasma or whole blood had no apparent effect on either folate assay.

Evidently, during zinc depletion there is a form of folate that is initially measured by RIA but then is converted to a metabolite that is poorly detected by RIA although still measurable microbiologically. Shane et al. (1) pointed out that most derivatives of folic acid that are metabolically important are equally measured microbiologically. In contrast, many forms of folate can give misleading results with RIA procedures because of different binding properties towards the binding protein used with the radioassay kits (1, 8).

Thus, in assessing the folate status of a patient by RIA, zinc nutriture must be considered as a nutrient that could produce falsely negative results. Further studies are in progress to establish the nature of these differences.

References
2. McGown EL, Lewis CM, Dong MH, Sauerberlich HE. Results with commercial radioassay kits compared with microbiological values.

<table>
<thead>
<tr>
<th>Dietary Zn*+, mg/day</th>
<th>Erythrocyte folate, µg/L</th>
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<tr>
<td></td>
<td>Folate-unsupplemented</td>
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<td>Microbiol.</td>
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<tr>
<td>8.0</td>
<td>312 ± 36 (2)*</td>
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<tr>
<td>3.5</td>
<td>353 ± 44 (17)</td>
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<tr>
<td>33.5</td>
<td>367 ± 67 (4)</td>
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*Mean ± SD (and no. of observations). b p < 0.001, Student’s Hest. f p < 0.004. f p < 0.05.

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“Improved” Sample Extraction before Liquid Chromatography of Prednisone and Prednisolone in Serum

To the Editor:

A recent Letter (1) reported on improvements on our extraction procedure for prednisone and prednisolone (2) by use of a commercially supplied column-type extraction tube (“Clin-Elut,” cat. no. 1003; Analytech Inc., International, Harbor City, CA 90710).

The sample extraction procedure proposed (1) was repeated by our group using two different batches of Clin-Elut tubes. We do agree that the extraction procedure was shortened, but we do not agree that the method was improved because, as demonstrated in Figure 1 (left), the chromatogram shows additional unidentified peaks and a delay in reaching the baseline after injection of the sample. A few injections of “Clin-Elut” extracts onto the column containing a 2-μm frit let the pressure rise from 1000 psi to an unacceptable pressure of 3000 psi. This plugging of the column was probably due to very fine particles eluted from the extraction tube. Thus we found it necessary to open the column in order before liquid chromatography of prednisone and prednisolone in human serum. Clin Chem 28, 2326–2327 (1982).


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Somatostatin in Diabetes

To the Editor:

Somatostatin, besides being of importance in the control of pituitary hormone release, may also have an important physiological role in the regulation of endocrine pancreas. The development of an immunoassay for somatostatin can contribute to an understanding of the pathogenesis of different types of diabetes mellitus. Only a few groups have reported values for man, and changes in somatostatin after some stimuli are not yet quite clear.

We have measured somatostatin in plasma in normal persons, in persons with impaired glucose tolerance (IGT), and in diabetic type II subjects after an oral glucose tolerance test (OGTT), according to the National Diabetes Data Group criteria (1), always with glucose basal concentrations <7.8 mmol/L.

Samples of whole blood were collected into pre-chilled tubes containing EDTA and aprotinin (Trasyol, Bayer), and the plasma was immediately separated by centrifugation (2000 × g, 10 min, 4°C). The plasma was stored at −80°C until use, then thawed in an ice bath.

We used an RIA kit to measure somatostatin (Immunonuclear Corp., Stillwater, MN 55082), involving an extraction procedure (acetone) and washing with organic solvent. The RIA was performed as described in the manufacturer’s literature.

For a group of 119 subjects, the mean basal reference value was 21.5 (SD 7.8) ng/L. The OGTT was performed in a group of 38 subjects (18 controls, 10 IGT, and 10 diabetes type II). In the three groups, the maximum increase among the basal values and those obtained during the OGTT were

Fig. 1. Dual-pen recordings of chromatograms (Right) For human plasma supplemented with prednisone and prednisolone, with use of the extraction procedure as described by Frey et al. (2). PO, prednisolone; HC, cortisol; D, dexamethasone (int. std.); P, prednisone; and C, cortisone. The attenuation of the upper pen is 10 times that for the lower pen recording. (Left) Same, but with use of the extraction procedure as described by Stewart et al. (1) to replace the frit after every 3rd to 6th injection.

The standard curves for prednisone and prednisolone were linear, as reported by Stewart et al. (1).

The method developed by our group (2) allows one to analyze simultaneously and specifically for prednisone, prednisolone, and the endogenous steroids cortisol and hydrocortisone (2). Stewart et al. (1), however, changed our conditions in such a way that for measuring prednisone and prednisolone “the interference from endogenous serum components such as cortisol and hydrocortisone was negligible” (1). However, as demonstrated for hydrocortisone on our chromatogram (Figure 1, right), endogenous cortisone and hydrocortisone are extracted by the method proposed by Stewart et al (1). These endogenous glucocorticoid concentrations may indeed not be negligible, as was recently shown in patients being treated chronically with prednisone (3).

We agree with the intention of Stewart et al. (1) that a simplified extraction procedure should be developed, but the modification of our method as proposed is inadequate because it shortens the extraction procedure at the expense of the quality of the chromatogram.

This work was supported by Grant 3.914-0-82 of the Swiss National Foundation for Scientific Research.

References
1. Stewart JT, Honigberg IL, Turner BM, Davenport DA. Improved sample extraction

2118 CLINICAL CHEMISTRY, Vol. 29, No. 12, 1983