CASE REPORT

Factitious transient neonatal hyperthyrotropinemia

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ABSTRACT. We report an infant with abnormally elevated levels of TSH determined in the Maryland State Laboratory for Neonatal Thyroid Screening, but normal levels in three other laboratories. The TSH level in the infant normalized by six months of age. The mother, who had a history of sarcoidosis, also had factitious hyperthyrotropinemia in the Maryland State Laboratory. Gel chromatography and ammonium sulfate precipitation of maternal serum demonstrated that the factor responsible for the factitious hyperthyrotropinemia was an immunoglobulin G. Maternal TSH levels in the Maryland State Laboratory were normalized by treatment of serum with polyethylene glycol. However, protein electrophoresis, immunoglobulin levels and immunofixation electrophoresis were all normal. We conclude that a subclass of immunoglobulins G, probably resulting from sarcoidosis, interfered with the precipitation of the TSH-antibody complex in the TSH radioimmunoassay of the Maryland State Laboratory.

INTRODUCTION
With the wide use of neonatal thyroid screening programs, several infants were reported to present factitious elevations of serum thyrotropin (TSH) (1-4). The factor responsible for this artifact from serum of neonates (1-4), as well as older subjects (5-7), was an immunoglobulin G (IgG) which bound to the primary antibody in various TSH radioimmunoassays (RIA), causing in turn a false elevation in the apparent amount of hormone. In neonates, these IgG's were acquired transplacentally and cleared from circulation within a few months. In some instances, they resulted from maternal exposure to animal proteins contained in vaccines (1, 6).

We studied an infant and her mother who had elevated levels of TSH by the filter paper assay of the Maryland Newborn Thyroid Screening Program, but normal levels in three other independent laboratories.

CASE REPORT
The patient was the full term product of a 20-year-old primigravida, delivered by cesarian section. The blood level of thyroxine (T4) at three days of age was 13.8 μg/dl (normal ≥ 7 μg/dl). A second determination of blood T4, routinely required at four weeks of age in the state of Maryland, was 3.4 μg/dl (normal ≥ 5 μg/dl). The blood levels of TSH assayed using the filter paper samples of three days and four weeks of age were 77.9 and 72 μU/ml, respectively (normal ≤ 21 μU/ml). Replacement therapy with L-thyroxine was begun at seven weeks of age, after obtaining a repeat blood sample. She was then evaluated in the Pediatric Endocrine Clinic of the Johns Hopkins Hospital, and at that time, the review of systems and physical examination of the infant were normal. The mother was clinically euthyroid and had no goiter. Her history was notable for an episode of pulmonary sarcoidosis when she was 17-year-old, which was documented by lung biopsy and was not felt to need treatment.

MATERIALS AND METHODS
Thyroxine
It was assayed by RIA in dried blood of a filter paper using the Immunospot T4 RIA (Meloy Laboratories, Springfield, VA), or in serum using T4 RIA kits from Biotex (Freswood, TX) or Travenol-Genentech (Cambridge, MA).

TSH
It was assayed by RIA using four different methodologies:
1) State of Maryland, Department of Health and Mental Hygiene, Neonatal Hypothyroidism Screening Laboratory. The sample was dried whole blood on filter paper.
collected by heel prick (8). The initial incubation mixture includes two 1/8 inch dots punched from the filter paper, bovine serum albumin, normal rabbit serum, human Chorionic Gonadotropin, [125I] TSH and high affinity rabbit antibody. After addition of 12.5% polyethylene glycol (PEG) solution, centrifugation and removal of the supernatant, the amount of radiolabel in the precipitate is determined in a gamma counter.

2) Bionetics (Kensington, MD). TSH was measured in serum by the HTSH RIABEAD Abbott Laboratory Kit (North Chicago, IL).

3) The Johns Hopkins Hospital. TSH was measured in serum by the Serono MAIA Monoclonal TSH3 Kit (BRAINTREE, MA).

4) The Virginia State Laboratory for Screening of Neonatal Hypothyroidism. TSH was measured in dried whole blood on filter paper using the Neonatal TSH Radioimmunoassay Kit from Becton and Dickinson Immunodiagnostics (Orangeburg, NJ).

TSH RIA of maternal serum following dilution with TSH-free serum
Aliquots of maternal serum were mixed with TSH-free serum (TSH undetectable in the HTSH RIABEAD assay) to produce 11 samples containing from 0 to 100% maternal serum. TSH was assayed in the Maryland State Laboratory using 50 µl aliquots of each dilution. The results were compared with the TSH standard curve generated in the Maryland State Laboratory for TSH assay.

TSH RIA of maternal serum following Sephacryl gel filtration
A 500 µl sample of maternal serum was chromatographed on a column (3.5 × 87 cm) containing Sephacryl S-300 gel (Pharmacon, Piscataway, NJ) equilibrated in phosphate buffered saline (PBS). Fifty drop elution fractions were collected and pooled to form three samples containing the 7S peak, the 19S peak, and intermediate material. The TSH concentration was assayed in the three samples at the Maryland State Laboratory.

TSH RIA of maternal serum following Ammonium Sulfate precipitation
Ammonium sulfate was added to 1 ml of maternal serum to make a 33% solution (w/v). After incubation at room temperature for 5 h, the precipitate was collected by centrifugation. The supernatant was removed and the pellet was resuspended in 1 ml of PBS. Precipitate and supernatant samples were dialyzed overnight in PBS, and each sample was adjusted to 1 ml. TSH was measured in 50 µl aliquots of supernatant and pellet samples at the Maryland State Laboratory.

TSH RIA of maternal serum following PEG precipitation
Aliquots of maternal serum were incubated at 4 C for 1 h with PEG (Sigma, St Louis, MO) and KET buffer, (2.5 M KCl, 0.05M EDTA and 10mM TRIS, pH 7.5) to produce 6 samples with PEG concentrations ranging from 0 to 20%. After centrifugation, 50 µl of each supernatant were used for TSH assay at the Maryland State Laboratory. The precipitates were collected, resuspended in KET buffer and 50 µl aliquots were also used for TSH assay. For control purposes, aliquots of serum from a patient with primary hypothyroidism (TSH = 646 µU/ml in the HTSH RIABEAD assay) were also processed as described above. In addition, aliquots of maternal and hypothyroid sera were incubated with KET buffer alone to produce ratios of serum to buffer that were identical to the ratios of serum to buffer in the PEG treated samples. These samples were processed in parallel with the PEG treated samples.

Additional studies on maternal serum
Maternal serum was subjected to serum protein electrophoresis (Helena, Beaumont, TX), immunoglobulin quantitation by nephelometric assay and immunofixation electrophoresis (Beckman, Brea, CA).

RESULTS
Table 1 shows the results of levels of T4 and TSH in the infant. Blood levels of TSH assayed at the Maryland State Laboratory were abnormally elevated at three days and four weeks of age. Blood T4 was normal at three days of age (13.8 µg/dl), but low (3.4 µg/dl, normal ≥ 5 µg/dl) at four weeks of age. However, when T4 determination was repeated using the dried blood sample obtained at four weeks of age, this repeat level was 6.4 µg/dl. When the infant was seven weeks of age, the blood level of TSH was abnormally elevated in the Maryland State Laboratory (62 µU/ml, normal ≤ 21 µU/ml), but serum TSH was normal (4.5 µU/ml, normal 0-5 µU/ml) in another laboratory. The blood level of TSH assayed at the Maryland State Laboratory declined to normal by six months of age. By contrast, all simultaneous serum TSH determi-

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<thead>
<tr>
<th>Age</th>
<th>T4 (µg/dl)</th>
<th>Normal values</th>
<th>TSH (µU/ml)</th>
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<tbody>
<tr>
<td>3 days</td>
<td>13.8&lt;sup&gt;1&lt;/sup&gt; (≥ 7)</td>
<td>78</td>
<td>-</td>
</tr>
<tr>
<td>4 weeks</td>
<td>3.4&lt;sup&gt;1&lt;/sup&gt; (≥ 5)</td>
<td>72</td>
<td>-</td>
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<tr>
<td>7 weeks</td>
<td>8.0&lt;sup&gt;2&lt;/sup&gt; (7.2-14.4)</td>
<td>62</td>
<td>4.5 (A)</td>
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<tr>
<td>11 weeks</td>
<td>12.4&lt;sup&gt;2&lt;/sup&gt; (7.2-14.4)</td>
<td>37</td>
<td>1.3 (B); &lt; 1 (C)</td>
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<tr>
<td>24 weeks</td>
<td>11.1&lt;sup&gt;2&lt;/sup&gt; (7.8-16.5)</td>
<td>&lt; 10</td>
<td>2.5 (B)</td>
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<sup>1</sup>Blood T4 and normal values from Maryland State Laboratory.
<sup>2</sup>Serum T4 and normal values from Ref. 9.
(A) = Bionetics; (B) = Johns Hopkins Hospital; (C) = Virginia State Laboratory.