Screening for Congenital Hypothyroidism by Assay for Thyrotropin in Dried Blood: Effect of Parenteral Nutrition

To the Editor:

In a recent Letter (1), Bourdoux et al. suggest that serum samples from neonates receiving parenteral nutrition may give falsely high values for thyrotropin (TSH) in congenital hypothyroidism (CH) screening programs based on either radiimmunoassay or two-site immunoradiometric assay (IRMA). Currently, all births in Scotland are screened by CH for use by an “in house,” two-site IRMA for TSH measurement in capillary blood dried onto filter-paper cards (2). Of the 150,000 infants screened to date, we have yet to find an artifactually increased value for the TSH ascribable to neonates being on parenteral nutrition, even though both Intralipid and Vamin Glucose (tested by Bourdoux et al. (1)) are used for intravenous nutrition of premature, malformed, and (or) sick neonates in Scotland.

To investigate the effect of these nutritional media in producing artifactually increased values for TSH, we assayed blood spots obtained from nine neonates being treated with Intralipid/Vamin glucose intravenously, using both the “in house” and a commercial (Immophase, Corning Medical and Scientific) two-site IRMA. Blood spots were also prepared to give a positive result (100 milli-int. units/L of whole blood) by adding TSH standard (MRC no. 68/38) or a negative result (0 milli-int. units/L of whole blood) in our screening protocol, for which our lower action limit is 25 milli-int. units/L of whole blood. Intralipid-20 or Vamin glucose (both from Kabé Vitrum) was added to give 0, 25, 50, 100, and 200 mL/L of whole blood. These specimens were then spotted onto filter cards (Schleicher and Schuell no. 2992) and allowed to air dry before analysis in replicate (n = 4) by the “in house” two-site IRMA.

Results obtained for the in vivo samples demonstrate that parenteral nutrition does not affect TSH values as measured by either assay method, because all samples gave values of 25 milli-int. units/L of whole blood. Furthermore, samples for one patient obtained before and during intravenous feeding were both below the action limit, confirming that parenteral nutrition did not produce increased results.

Similarly, addition of Vamin Glucose up to 200 mL/L of whole blood did not significantly affect expected hormone values (mean recovery 99.5%) (Table 1). Adulteration with Intralipid, however, resulted in a smaller observed recovery (mean 84.5%) than control values. Although the latter results are significantly reduced (p < 0.01, Student’s t-test) the observed reduction only becomes important with TSH values close to the action limit, where a value of 28 milli-int. units/L of whole blood would be analyzed as 24 milli-int. unit/L. However, all proven cases of primary hypothyroidism we have detected to date (n = 39) have shown TSH values of greater than 50 if not 100 milli-int. units/L of whole blood and thus this artifactual effect may not be clinically significant.

These results obtained for the two-site IRMA are not compatible with those of Bourdoux et al. (1), who found serum TSH results to be increased both in the Corning assay and some radioimmunoassay systems. These investigators proposed that the possible analytical interference occurred either at the level of antiserum specificity or the separation system, favoring the latter possibility. However, interference with the separation system of the two-site IRMA is not probable because the specific antibodies are covalently immobilized onto a solid-phase matrix, unlike the liquid-phase radioimmunoassays. However, any substance which nonspecifically interferes with the antigen–antibody reaction will cause a decreased specific binding of labeled (or unlabeled) antigen. With the limited reagent radioimmunoassay, decreased labeled antigen binding will be interpolated as increased hormone values. However, the two-site IRMA is a reagent-excess assay (3), where increasing bound antigen gives an increasing signal and binding of antigen reduced by physical interference will result in an apparently lower hormone value. This is demonstrated with Intralipid adulteration, where the observed hormone results are 15% less than expected. As one of the “negative” samples had lower binding than the 0 standard, the effect is probably one of interference with the antibody–antigen reaction, and is probably nonspecific in nature, because there is no apparent relation between the proportion of adulteration and the reduction in observed values (Table 1).

The apparent discrepancy between our observations and those of Bour-

<table>
<thead>
<tr>
<th>Table 1. Values Observed for TSH in Dried Blood Samples Adulterated with Parenteral Nutrition Media</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
<tr>
<td><strong>Adulterant</strong></td>
</tr>
<tr>
<td>Vamin</td>
</tr>
<tr>
<td>0</td>
</tr>
<tr>
<td>100</td>
</tr>
<tr>
<td>Intralipid-20</td>
</tr>
<tr>
<td>0</td>
</tr>
<tr>
<td>100</td>
</tr>
</tbody>
</table>

*Significantly less than control at p < 0.01

UD = less than 5 milli-int. units/L whole blood

P. P. Kamoun
Ph. Parvy
L. Cathelineau

Lab. de Biochimi, Genétique
Hôpital Necker-Enfants Malades
75743 Paris Cedex 15, France

difficult
diabetic
Scotland
Royal
Glasgow
1-72.
nonat
tropic
et.
I.
Nevertheless,
Ekins
heur
Beta-
Einth
from
The
inconclusive,

equently,
erythropoiesis,
congental
2. Sutherland RM, Ratcliffe JG, Chapman RS. Immunoassay of blood spot TSH; develop-
ment of a rapid two-site immunoradiometric assay and comparison with radio-
3. Ekins RP. General principles of hormone assay. In Hormone Assays and Their Clinical
Application, JA Lorsie, ET Bell, Eds., Churchill Livingston, Edinburgh, 1976, pp
1–72.

Randal M. Sutherland
Royal Infrmary
Glasgow G4 OSF
Scotland
Helen J. Jackson
Dept. of Med. Genetics
Royal Hosp. for Sick Children
Glasgow
Scotland

The first author of the Letter in question responds:

To the Editor:

Neither of the two commercial as-
says (of which one was an immuno-
radiometric assay) for which we observed
large interferences was tested by these
authors.

Using the Immophase assay from
Corning we observed a small interfer-
cence (Vamin Glucose 7.6. and Intra-
lipid 2.2. milli-int. units/L of serum), val-
cues clearly below the detection limit
(15 milli-int. units/L of whole blood = 30 milli-int. units/L serum) reported by
these authors for the Corning assay.

In this condition, any interference is
difficult to demonstrate. Nevertheless,
a large discordance appears for patient
2: in-house assay: 7 milli-int. units/L of
whole blood (= 14 milli-int. units/L
serum) and Corning assay 16 milli-int.
units/L of whole blood (= 32 milli-int.
units/L serum).

Thus the information provided by
Sutherland and Jackson does not in-
validate the data reported in our study.
Their argument relies on theoretical
principles that have not been verified.

The mechanisms of these interfer-
ences are still open to discussion and
deserve further investigation.

P. Bourdoux
Universit{233} Libre de Bruxelles
1000 Bruxelles, le Rue Haute, 322
Belgium

To the Editor:

Our work was not supposed to be an
assessment of a large number of com-
mercial kits with whose overall assay
characteristics and utility we were not
familiar, but a deeper analysis, using a
single assay system with which we
have extensive experience. We think
this is a more useful exercise, adding
relevant information to the authors’
broad observations.

Our conclusions are based on both
the theoretical and practical aspects of
TSH isotopic immunoassays, which
have been well established by our-
selves (ref. 2, above) and follow the
general lines of definitive publications
in this area (e.g., ref. 3. Ekins RP. The
future development of immunoassay.
In Radioimmunoassay and Related
Procedures in Medicine, 1, Berlin,
275). Thus the authors’ comments con-
cerning our broad analytical bases are
not valid.

Similarly, the significance of the
authors’ comments concerning the detec-
tion limit must also be questioned,
because the level of interference ob-
erved (7.6. and 2.2. milli-int. units/L of
serum) is not significant in screening
terms, nor in our experience are values
of 16 or 17 milli-int. units/L of whole
blood, where normal ranges extend up
to 25 milli-int. units/L whole blood and
confirmed positive results invariably
exceed 100 milli-int. units/L of whole
blood.

We believe our conclusions and dis-
cussions are justified and that paren-
teral nutrition, in our experience, is
not a significant factor in misclassifica-
tion of screening results.

Randal M. Sutherland
Helen J. Jackson

Urnalysis for Blood: Questionable Interpretation of Reagent Strip
Results

To the Editor:

Currently, we investigate hematuria
and hemoglobinuria by means of simple
urinalysis reagent strips, Hematostix1
and Labstix1 (Ames Division, Miles
Miles Laboratories). These rely on the
peroxidase-like activity of hemoglobin
to catalyze the reaction between o-
tolidine and cumene hydroperoxide to
produce a blue-colored compound.
They are also widely used in wards and
outpatient departments by the nursing
staff as a rapid screening test. Recent
papers (1, 2) have pointed out that strips
containing o-tolidine may give false-negative results if a high con-
centration of ascorbate is present in the
urine; others (3) note that false posi-
tives may arise from the presence of
bacterial enzymes. Indeed, Escherichia
coli reportedly (4) contains o-tolidine
peroxidase, the substrate being structurally similar to o-tolidine.

An unexpectedly high incidence of
positive findings on strip testing for
blood in urines from a urological outpa-
tient department in this hospital led us
to suspect that the test materials were
being handled improperly. Alterna-
tively, we thought that positive results
were being accepted at face value with-
out due regard to the possibility of
interferences such as those suggested
by the manufacturer. Although we
took steps to ensure correct use of the
strips, many apparent positive results
continued to be obtained. This problem
prompted us to consider methods of
improving the diagnostic utility of the
strips.

Standard texts (5) suggest that per-
oxidases are destroyed on strong heat-
ing, whereas hemoglobin peroxidase
activity is not. However, other sources
revealed a possible decrease in hemo-
globin enzymatic activity on heating
(6), and that certain vegetable peroxi-
dases are activated at 70 °C (7). In
addition, practical experience showed
that strip tests on positive urine con-
trols remained positive after the urine
was boiled if the hemoglobin concen-
tration exceeded about 300 µg/L. Fur-
ther attempts to find a discriminatory
inhibitor included tests of the com-
pounds listed in Table 1, added to urine
containing hemoglobin and vegetable
or bacterial peroxidases (Table 1). Only
solid zinc sulfate, added to give a con-
centration of approximately 50 mg/mL,
inhibited the hemoglobin enzymatic
activity significantly with negligible
effect on the peroxidase control sam-
ples. Application of this potential dif-
terentiation method to patients’ urines
has as yet been inconclusive, and
urines generally are further investi-