Plasma P_I and Erythrocyte 2,3-Diphosphoglycerate Concentrations of Non-Acidotic Diabetics in Various Degrees of Metabolic Control

To the Editor:

Recently Kalofoutis et al. (1) suggested that erythrocyte 2,3-diphosphoglycerate (2,3-DPG) concentration in diabetics is the same as in normal persons. However, others find that in diabetics it fluctuates in relation to the state of metabolic control, not only during ketoacidosis and recovery when initially the erythrocyte 2,3-DPG content is markedly depressed (2, 3), but also in non-acidotic patients (4, 5). We have attempted to assess whether variation of plasma inorganic phosphate (P_i) affects the erythrocyte 2,3-DPG concentration in non-acidotic diabetics as it does in patients recovering from severe ketoacidosis (3).

Table 1 presents results for P_i and erythrocyte 2,3-DPG measured in a group of 10 non-acidotic, newly diagnosed, and otherwise healthy youth-onset type diabetics (8 males, 2 females; age range, 15 to 40 years) on the day of hospital admission, before insulin treatment (Group I); on the day after insulin therapy had been started (Group II); and on the day of best metabolic control (Group III). 2,3-DPG exhibited the same fluctuating pattern as P_i, and in fact was correlated with P_i for all measurements performed in the diabetics on a total of 40 days (Figure 1).

Altered P_i in diabetic subjects may originate from several mechanisms: metabolic damage, and hemodilution, variation in urinary loss according to the degree of glycosuria, and (or) changes in cellular uptake of P_i in relation to glucose, especially by insulin-sensitive tissues. For all these reasons diabetics are highly susceptible to variations in the phosphate pool available for the erythrocytes. In the erythrocyte itself P_i is likely to influence 2,3-DPG concentration by its effect on 6-phosphofructokinase (EC 2.7.1.11) (6) and glyceraldehyde-phosphate dehydrogenase (EC 1.2.1.12) (7).

Hence, and as our results indicate, during changing metabolic conditions of diabetes P_i seems to be a strong determinant of erythrocyte 2,3-DPG content. Obviously the metabolic state of diabetes has to be considered, even in non-acidotic patients, before a reliable statement can be made on the amount of 2,3-DPG in diabetics. Depending on our selection criteria for patients, we could have reported 2,3-DPG values to be normal, decreased, or increased in diabetics. The conflict of data in the literature concerning erythrocyte 2,3-DPG concentration in non-acidotic diabetics (1, 4, 5, 8-10) therefore, seems likely to be attributable to differences in metabolic control.

Apparently it is important that erythrocyte 2,3-DPG concentrations can be low in non-acidotic diabetics in some metabolic states and that the oxygen-delivering capacity of erythrocytes is probably concurrently impaired in such patients.

Table 1. Some Clinical Chemical Values in 10 Newly Detected, Non-acidotic Diabetics at Various Stages of Metabolic Control and in 15 Normal Controls

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Hemoglobin, g/100 ml</th>
<th>Blood glucose, mmol/liter</th>
<th>P_i, mmol/liter</th>
<th>2,3-DPG, μmol/g Hb</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diabetics</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>7</td>
<td>15.5 ± 1.4</td>
<td>16.1 ± 4.4</td>
<td>1.08 ± 0.11</td>
<td>14.94 ± 1.40</td>
</tr>
<tr>
<td>II</td>
<td>10</td>
<td>15.1 ± 1.5</td>
<td>11.9 ± 1.9</td>
<td>0.98 ± 0.14</td>
<td>13.10 ± 1.46</td>
</tr>
<tr>
<td>III</td>
<td>10</td>
<td>14.3 ± 1.2</td>
<td>6.1 ± 1.4</td>
<td>1.20 ± 0.10</td>
<td>16.53 ± 1.19</td>
</tr>
<tr>
<td>Normal controls</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IV</td>
<td>15</td>
<td>14.2 ± 1.1</td>
<td>5.1 ± 0.9</td>
<td>1.13 ± 0.10</td>
<td>14.40 ± 1.28</td>
</tr>
<tr>
<td>Significance (P-values) between groups</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P_i</td>
<td>2,3-DPG</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group</td>
<td>I II III IV</td>
<td>I II III IV</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>&lt;0.005 &lt;0.05 N.S. d</td>
<td>N.S. &lt;0.02 &lt;0.01 N.S.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>&lt;0.005 &lt;0.01 N.S.</td>
<td>&lt;0.001 &lt;0.05</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>N.S.</td>
<td>&lt;0.001 d</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a N.S., nonsignificant.

References
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Thyroid-function Tests in
Diphenylhydantoin-treated Patients

To the Editor:
A recent article in your journal (1)
made two important points: that ther-
apeutic concentrations of diphenylhy-
dantoin in serum do not affect the
binding of thyroxine to serum pro-
enas, and that a free-thyroxine index
may not be a valid indicator of thyroid
status in diphenylhydantoin-treated
patients. The authors found that by
the Ames method 21% of patients test-
ed had free-thyroxine indices that
were within the hypothyroid range,
and that by the Murphy–Pattee meth-
ods the mean free-thyroxine index was
significantly lower than in normal
controls, although none of the individ-
ual values were within the hypo-
roid range.

We believe that the true results are
more striking than this, because the
authors have not used their own refer-
ence ranges, but rather those of the
manufacturer of the kits and of a dis-
tant laboratory. If one assumes the
distributions of serum total thyroxine
concentration and free-thyroxine
index to be gaussian and uses the au-
tors’ data, the 95% reference ranges
(±2 SD) for these two measurements
are respectively 44–84 μg/liter and
44–84 for the Ames and 54–94 μg/liter
and 53–90 for the Murphy–Pattee
method. With these reference ranges,
about 40–50% of diphenylhydantoin-
treated patients have low serum total
thyroxine concentrations and free-
thyroxine indices by both techniques.

This illustrates the necessity to use
reference ranges that have been deter-
mind in the population from which the
tested patients are drawn. Perhaps
laboratories performing tests on speci-
mens received from a distance should
point out that their own quoted refer-
ence ranges may not necessarily apply
to the specimens tested. We know of
one study in which specimens were
sent to a laboratory 2500 miles away
for serum total thyroxine assay and
the use of the laboratory’s quoted ref-
ence range led to incorrect conclu-
sions (2); we have written to the au-
tors to this effect.

In the original report on the effect
of diphenylhydantoin on thyroxine
binding to serum proteins (3), phar-
macological drug concentrations were
used, and if one extrapolates the data
to a therapeutic diphenylhydantoin
decentration of 15 mg/liter in the
serum tested, the mean effect on
erythrocyte triiodothyronine uptake
was less than 6%.

In the study under discussion, in
the Ames method, therapeutic concen-
trations of diphenylhydantoin in
serum added to the Sephadex column
did not affect triiodothyronine up-
take. However, if an equivalent
amount of the drug was first added to
the column and serum then tested, tri-
odothyronine uptake was increased by
35%. We were puzzled by these ap-
parently conflicting observations.

Other workers (4) have found high
serum thyrotropin hormone and low
serum total triiodothyronine concen-
trations in diphenylhydantoin-treated
patients, whereas the authors of this
study found normal serum thyrotropin
hormone concentrations and would presumably predict high serum total
triiodothyronine concentrations
because of increased peripheral con-
version of thyroxine to triiodothyro-
nine. The reasons for these discrep-
ancies are not clear.

The major finding of this study is
obviously of great importance: nearly
half of apparently euthyroid diphenyl-
hydantoin-treated patients will have
“hypothyroid” serum total thyroxine
concentrations and free-thyroxine in-
dices when compared to nontreated
controls. Thus, alternative measure-
ments (e.g., of serum thyrotropin hor-
mon concentration) are necessary to
evaluate thyroid status. We await with
interest further reports on the effects
of diphenylhydantoin on thyroid-
function tests.

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The authors of the paper in question
respond:

To the Editor:
We thank Drs. Phillips and Pain for
their critical observations, which are
in accord with our stated views re-
garding the “hypothyroid” total thy-
roxine concentration and free thyrox-
ine indices” in diphenylhydantoin-
treated patients. We agree that the
comparison between our controls and
the patients is even more striking if
the 95% reference ranges (±2 SD) are
used. We elected not to do this be-
cause of the small number of normal
controls. Instead, we used the manu-
facturer’s range to emphasize that
such a range is not valid for patients
being treated with diphenylhydantoin.

Therapeutic concentrations of
diphenylhydantoin in serum have no
practical effect upon the triiodothyro-
nine uptake test. In the experiment in
which diphenylhydantoin was pre-
loaded on Sephadex columns, the con-
titions differed from those of the rou-
tine assay. Some of the labeled diphe-
nylhydantoin was eluted from the Se-
phadex column by the serum, presum-
ably because of protein binding, when
the column had been preloaded with
diphenylhydantoin. Whether the am-
ount of diphenylhydantoin dis-
placed from the Sephadex is accessible
to samples of serum and becomes
“more free” to displace labeled tri-