entirely adequate, for the following reasons: (a) in none of these studies were there sufficient numbers of healthy children in the various age groups; (b) The methodology varied considerably, with two consisting of bi-directional paper chromatography; and (c) three of the studies were based on 24-h collection of urine specimens, an almost impossible requirement in infants and young children.

We therefore elected to evaluate a reasonable number of urine samples from healthy children of different age groups by Pisano's method (7), because it appears to be the most reliable spectrophotometric technique in common use. Urine samples, primarily from children admitted for elective surgery, were collected exactly as for HVA (2). Because of the difficulty in obtaining 24-h urine samples, as well as for the convenience of the patient and the laboratory, we measured VMA in casual (i.e., untimed) specimens and calculated the results in micrograms per milligram creatinine (Table 1). As one might predict, the concentration is highest for infants and decreases with increasing age, reaching the lowest values in the 10–15 year age group.

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References


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Variation of Trace Binding in Digoxin Radioimmunoassay by Use of 125I-Labeled 3-O-Succinyl-Digoxigenin: Relation of Thyroxine Concentration to Binding

To the Editor:

Kroening et al. (1) recently showed that between-sample variation in tracer binding in the 125I-labeled digoxin radioimmunoassay was greater for 3-O-succinyl-digoxigenin-[125I]thyroxine than for the 125I-thyroxine-methyl-ester digoxin. Specifically, they found the percentage of tracer bound to be significantly increased for samples with low thyroxine concentration when assayed with the first tracer, as compared to samples with normal or high thyroxine concentration. Little difference existed when the latter tracer was used. Also, they showed variation in tracer binding when serum from dogs dosed with thyrotropin was assayed with the first tracer, but found little variation with the second. They concluded that tracer binding was influenced by thyroxine-binding proteins. Other studies (2) have also shown variation in binding with different plasmas.

In a brief study of the Becton-Dickinson solid-phase radioimmunoassay for digoxin, we found little difference in tracer binding, whether the patient was hypothyroid, euthyroid, or hyperthyroid. The following tabulation shows data for some of these patients as well as their thyroxine, T-3 uptake, and free thyroxine index (T-7) values.

<table>
<thead>
<tr>
<th>Binding of tracer, %</th>
<th>T-4 uptake, %</th>
<th>T-3 uptake, %</th>
<th>Free thyroxine index</th>
</tr>
</thead>
<tbody>
<tr>
<td>54.2</td>
<td>28</td>
<td>28</td>
<td>0.48</td>
</tr>
<tr>
<td>55.5</td>
<td>30</td>
<td>30</td>
<td>0.76</td>
</tr>
<tr>
<td>54.4</td>
<td>33</td>
<td>33</td>
<td>0.72</td>
</tr>
<tr>
<td>56.9</td>
<td>54</td>
<td>54</td>
<td>8.80</td>
</tr>
<tr>
<td>55.1</td>
<td>61</td>
<td>61</td>
<td>12.24</td>
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<tr>
<td>54.3</td>
<td>62</td>
<td>62</td>
<td>13.26</td>
</tr>
<tr>
<td>54.9</td>
<td>40</td>
<td>40</td>
<td>2.94</td>
</tr>
<tr>
<td>54.2</td>
<td>39</td>
<td>39</td>
<td>2.86</td>
</tr>
</tbody>
</table>

Unlike Kroening et al. (1), we are unable to demonstrate any significant difference in the binding of the 3-O-succinyl-digoxigenin-[125I]thyroxine tracer with different sera. They speculate that the variation in binding seen with this tracer, in contrast to the other derivative, is related to the molecular configuration or the presence of the digitoose sugars, resulting in less nonspecific binding. Our data, obtained with the use of the solid-phase radioimmunoassay procedure, suggest that lack of specificity or antibody affinity of the antisera may be the cause of variation in tracer binding with variations in sample thyroid-binding globulin concentration.

References


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Improved Gas-Chromatographic Analysis for Anticonvulsants

To the Editor:

We report here our experience with the packing SP 2510 DA and use of two internal standards for measuring therapeutic concentrations of some commonly determined anticonvulsant drugs (phenobarbital, PB; carbamazepine, CZ; primidone, PD; phenytoin, DPH), underivatized, by gas-chromatography.

Individual stock standards of, per liter of methanol, 800 mg of PB, 1000 mg of CZ, 800 mg of PD, and 800 mg of DPH are prepared. Methanolic working standards are prepared from the stock standards to contain the following concentrations (mg/liter): 10.0 PB, 2.0 CZ, 2.0 PD, and 10.0 DPH for subthera-
peutic; 20.0 PB, 10.0 CZ, 8.0 PD, and 20.0 DPH for therapeutic; 40.0 PB, 15.0
CZ, 20.0 PD, and 40.0 DPH for above-therapeutic. A combined internal stan-
dard is prepared containing, per liter, 125 mg of 5-methyl-5-phenylhydantoin
(MPH) and 250 mg of 5-p-(methyl-
phenyl)-5-phenylhydantoin (MPPH). 
These solutions are all stable for at least 10 months when stored in Teflon-lined 
screw-top glass containers at −40 °C. 

The lack of commercially available, 
reliable control material for anticon-
vulsant drugs (1) prompted us to con-
sider a self-prepared control. Hemphill 
reported that a serum pool supple-
mented with pure drugs was stable at
−76 °C for at least 1 year (Hemphill G., 
Panel discussion, AACC Midwest Sec-
tion Meeting, Springfield, Mo., April 29, 1977); therefore we prepared a control
by the following technique. Solutions 
approximating 20 mg/liter PB, 6 mg/
liter CZ, 10 mg/liter PD, and 20 mg/liter
DPH are prepared in 50 ml of acetone. 
A 0.1 ml aliquot is removed and ana-
alyzed in duplicate to confirm target 
values. The balance is transferred to 
500-ml volumetric flask with four ace-
tone washes and evaporated in a stream of 
air, which is filtered (5-μm filter, Type 
620-4H air filter; Watts Regulator Co., 
Lawrence, Mass. 02116) and then dried 
by passage through a magnesium per-
chlorate-packed polyethylene drying 
column (no. 19962; Bel-Art Products, 
Pequannock, N.J. 07440) with a 4-cm 
plug of indicating Drierite (W.A. Ham-
mond Drierite Co., Xenia, Ohio 45385) 
at the exit. Drug-free pooled plasma is 
then added and the mixture is stirred for 
2 h at room temperature; 1.5-ml portions 
are placed in sterile polyethylene snap-
top tubes, frozen, and stored at −75
°C. 

For the extraction, 100 μl of internal 
standard is added to each Teflon-lined 
screw-top glass extraction tube (16 × 
100 mm); 1.0 ml of each working standard 
is added to its appropriate tube. The 
contents of all tubes are evaporated at 
room temperature in a stream of dry, 
filtered air. Drug-free plasma, 1.0 ml, is 
added to all standard tubes, 1.0 ml of 
control pool or patient serum to all other 
tubes. The pH is adjusted to about 2 by 
adding four drops of 3 mol/liter hydro-
chloric acid. 

The acidified serum is vortex-mixed 
and then extracted by hand with 6.0 ml 
of methylene chloride, with a gentle 
rocking motion, for 2 min. The capped 
tubes are spun at 3000 rpm for 2 min in
an explosion-proof centrifuge. The 
acid layer is aspirated and discarded and 
the organic (lower) layer is trans-
f erred to a 5-ml conical test tube and 
evaporated. The drug residue is reconstituted with 0.2 ml of acetone and 
vortex-mixed. The acetone solution is 
transferred to a glass vial and capped with 
Teflon-lined aluminum seals (vials 
no. 223682 and caps no. 224211; Wheat-

Our Perkin-Elmer Model 3920B gas 
chromatograph is equipped with a heated flash vaporizer injector and 
flame ionization detector. A glass-lined 
receiver tube is used between the col-
um and detector for an all-glass sys-
tem. The 184 cm × 4 mm i.d. glass 
column packed with GP 2% SP 2510 DA on 
100/120 Supelcoport (support or pre-
packed columns obtained from Supelco, 
Inc., Bellefonte, Pa. 16823) is condi-
tioned by the Supelco recommended 
procedure (2). 
The column is programmed from 220 
°C to 265 °C, at 16 °C/min with an initial 
hold of 0 min and a final hold of 16 min. 
(Although a final hold of only 12 min is 
necessary, 16 min was predicated by 
instrumental parameters.) The program 
is started simultaneously with manu-
ual sample injection via a 10-μl syringe 
(Hamilton Co., Reno, Nev. 89510) or an 
automatic sampling device (Model 4900; 
Precision Instruments, Baton Rouge, 
La. 70895). 
The chromatographic conditions are:

<table>
<thead>
<tr>
<th>Carrier gas:</th>
<th>N2</th>
</tr>
</thead>
<tbody>
<tr>
<td>inlet press:</td>
<td>483 kPa (70 psi)</td>
</tr>
<tr>
<td>flow rate:</td>
<td>75 ml/min</td>
</tr>
<tr>
<td>Hydrogen pressure:</td>
<td>166 kPa (24 psi)</td>
</tr>
<tr>
<td>Air pressure:</td>
<td>252 kPa (35 psi)</td>
</tr>
<tr>
<td>Injector temp.</td>
<td>275 °C</td>
</tr>
<tr>
<td>Detector temp.:</td>
<td>325 °C</td>
</tr>
<tr>
<td>Attenuation:</td>
<td>128</td>
</tr>
<tr>
<td>Range:</td>
<td>1</td>
</tr>
<tr>
<td>Sample vol.:</td>
<td>5 μl</td>
</tr>
</tbody>
</table>

Figure 1 illustrates a typical chro-
matogram obtained for a control ma-
terial. The relative retention times for 
the peaks shown in Figure 1 are: 0.30 for 
MPH, 0.32 for PB, 0.38 for CZ, 0.60 for 
PD, 0.87 for DPH, and 1.00 for MPPH. 
An unidentified component, shown as 
peak A in Figure 1 and described in the 
literature as a plasticizer (2), has a rela-
tive retention time of 0.21. Phenyl-
methylmalonamide (PEMA), a metab-
olite of PD, elutes after peak A and has 
a relative retention time of 0.23. Nor-
mesantoin (5-ethyl-5-phenylhydantoin), 
a metabolite of mesantoin, elutes on the 
leading edge of PB with a relative re-
etention time of 0.315. Chromatograms 
for patients’ samples and for standards 
are identical to the control. We use MPH 
as an internal standard for PB, CZ, and 
PD, and MPPH as an internal standard 
for DPH. Quantitation is by peak-height 
ratios. Each chromatographic run con-
ists of subtherapeutic, at above-
therapeutic standards, patients’ 
samples, and a control pool. A standard 
curve of peak-height ratio vs. concen-
tration is plotted for each drug. Stan-
dard curves for PB and DPH are linear to 
least 80 mg/liter and have regression 
equations of y = 10.3x + 0.2 and y =

19.8x + 0.4 (n = 175), with standard 
error of estimates of 0.21 and 0.42, re-
spectively. Those for CZ and PD are 
linear to at least 30 mg/liter and have 
regression equations of y = 18.9x + 0.5 
and y = 25.7x + 0.04 (n = 168), with 
standard error of estimates of 0.24 and 
0.85, respectively. The regression 
equations were calculated by using 
non-weighted least-squares, excluding 
the zero intercept. Although linearity 
does extend beyond our working range, 
we dilute with distilled water any speci-
men which exceeds the above-thera-
peutic standard, re-extract, and 
rechromatograph. 

Our prepared control material was 
used to estimate the within-day preci-
sion. The results (mean ± 2 SD) were:

<table>
<thead>
<tr>
<th>Drug</th>
<th>Mean ± 2SD mg/liter</th>
</tr>
</thead>
<tbody>
<tr>
<td>PB</td>
<td>20.3 ± 0.88</td>
</tr>
<tr>
<td>CZ</td>
<td>6.9 ± 0.66</td>
</tr>
<tr>
<td>PD</td>
<td>9.2 ± 0.92</td>
</tr>
<tr>
<td>DPH</td>
<td>20.0 ± 0.80</td>
</tr>
</tbody>
</table>

(No. 10). Day-to-day precision of the 
control pool during five months (mean ± 
2SD) was: 20.0 ± 1.39, 6.5 ± 0.87, 10.2 ± 
0.96, and 19.9 ± 1.32 mg/liter for the 
respective drugs (n = 49). Thus, target 
values and control pool values agreed 
well.

Analytical recovery was evaluated by 
supplementing a patients’ specimen 
with a known amount of pure drug and 
carrying duplicate samples through the 
complete procedure. Percentage recov-
eries were 100.3 for PB, 100.0 for CZ, 
101.7 for PD, and 98.4 for DPH. Ex-
traction efficiencies were determined by 
comparing peak-height ratios for the 
therapeutic drug standard extracted from 
a drug-free pool to the peak-height 
ratios of the same volume of unextracted 
standard in methanol. The methylene 
chloride extract of the serum-based
standard and the unextracted standard was supplemented with internal standard before evaporation and reconstitution with acetone as suggested by Gupta et al. (3). Extraction efficiencies (percent) were 90 for PD, 98 for C2, 69 for D, and 86 for DPH, which agrees favorably with results of other workers (4). Heipertz et al. (5) indicated that the extraction of primidone can be improved by combining a second extract, including ammonium sulfate, to the original extract. We confirm their findings, but our data indicate that a single extraction is adequate for monitoring therapeutic concentrations of these drugs.

Cholesterol interferes with measurement of PD when SP 2510 DA packing material is used on a 92-cm column (2), but it can be separated from primidone by using a short precolumn of 5% SP 2250 (4). With our recommended procedure and column, this contaminant can only be detected after several hundred injections, and replacing the initial 10 to 15 cm of packing monthly has eliminated this serum component from our chromatograms. Supelco indicates that PEMA elutes at the same time as a common plasticizer (2). Our recommended procedure completely resolves these components, thus allowing PEMA to be quantitated. 5-Ethyl-5-phenylhydantoin is eluted on the leading edge of phenobarbital and visual inspection of the chromatogram allows for the interpretation of this interfering component. We have encountered no known interfering substances which cannot be identified.

References

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Evaluation of the Space Stat 30
Sodium/Potassium Ion Analyzer

To the Editor:
We evaluated a new sodium/potassium ion analyzer, the Space Stat 30 (SS30) made by Orion Biomedical (and available in the U.K. from M.S.E. Scientific Instruments, Manor Royal, Crawley, West Sussex) and compared the results with those obtained with the Corning EEL 450 and Technicon SMA 6/60 Flame photometers.
The SS30 is a two-channel potentiometric automated system for measuring sodium and potassium in (a) separated plasma and (b) the plasma of whole blood. It incorporates a sodium electrode with a sodium permeable glass membrane, a potassium sensor with a porous organophilic membrane saturated with liquid ion exchanger, and a reference electrode, all maintained at 37 ± 1 °C. The plasma or whole-blood sample, 0.5 ml, is injected, and is delivered to the electrodes by a peristaltic pump. Operational instructions, error warnings and results of analyses are displayed. Reagents and standard so-

<table>
<thead>
<tr>
<th>Table 1. Precision of SS30</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mean value</strong></td>
</tr>
<tr>
<td><strong>Sodium</strong></td>
</tr>
<tr>
<td>Within-batch (n = 20)</td>
</tr>
<tr>
<td><strong>Aqueous sample</strong></td>
</tr>
<tr>
<td>117.4</td>
</tr>
<tr>
<td>152.9</td>
</tr>
<tr>
<td>162.3</td>
</tr>
<tr>
<td><strong>Plasma</strong></td>
</tr>
<tr>
<td>135.9</td>
</tr>
<tr>
<td>143.6</td>
</tr>
<tr>
<td>158.9</td>
</tr>
<tr>
<td><strong>Blood</strong></td>
</tr>
<tr>
<td>133.7</td>
</tr>
<tr>
<td>140.5</td>
</tr>
<tr>
<td>164.7</td>
</tr>
<tr>
<td>Between-batch (n = 20)</td>
</tr>
<tr>
<td><strong>Aqueous sample</strong></td>
</tr>
<tr>
<td>115.6</td>
</tr>
<tr>
<td>143.0</td>
</tr>
<tr>
<td>156.0</td>
</tr>
<tr>
<td><strong>Plasma</strong></td>
</tr>
<tr>
<td>117.0</td>
</tr>
<tr>
<td>135.6</td>
</tr>
<tr>
<td>158.8</td>
</tr>
</tbody>
</table>

Table 2. Comparison of Results for 100 Patients' Specimens Analyzed with the SS30, SMA 6/60, and Corning EEL Flame Photometer *

<table>
<thead>
<tr>
<th>y</th>
<th>x</th>
<th>Slope m</th>
<th>Intercept c</th>
<th>Mean y result</th>
<th>Mean x result</th>
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</thead>
<tbody>
<tr>
<td><strong>Sodium</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SS30</td>
<td>SS30</td>
<td>0.951</td>
<td>0.882</td>
<td>+4.751</td>
<td>143.3</td>
</tr>
<tr>
<td>SS30</td>
<td>6/60</td>
<td>0.892</td>
<td>0.768</td>
<td>+36.588</td>
<td>143.3</td>
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<tr>
<td>SS30</td>
<td>EEL</td>
<td>0.893</td>
<td>0.805</td>
<td>+32.898</td>
<td>143.3</td>
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<tr>
<td>SS30</td>
<td>6/60</td>
<td>0.896</td>
<td>0.764</td>
<td>+35.111</td>
<td>141.2</td>
</tr>
<tr>
<td>SS30</td>
<td>EEL</td>
<td>0.899</td>
<td>0.783</td>
<td>33.970</td>
<td>141.2</td>
</tr>
<tr>
<td>EEL</td>
<td>6/60</td>
<td>0.943</td>
<td>0.875</td>
<td>+15.336</td>
<td>137.0</td>
</tr>
<tr>
<td><strong>Potassium</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SS30</td>
<td>SS30</td>
<td>0.990</td>
<td>0.996</td>
<td>+0.105</td>
<td>4.17</td>
</tr>
<tr>
<td>SS30</td>
<td>6/60</td>
<td>0.958</td>
<td>1.018</td>
<td>+0.084</td>
<td>4.17</td>
</tr>
<tr>
<td>SS30</td>
<td>EEL</td>
<td>0.951</td>
<td>0.933</td>
<td>+0.266</td>
<td>4.17</td>
</tr>
<tr>
<td>SS30/Plasma</td>
<td>6/60</td>
<td>0.977</td>
<td>1.025</td>
<td>-0.028</td>
<td>4.08</td>
</tr>
<tr>
<td>SS30/Plasma</td>
<td>EEL</td>
<td>0.977</td>
<td>1.014</td>
<td>+0.100</td>
<td>4.08</td>
</tr>
<tr>
<td>EEL/Plasma</td>
<td>6/60</td>
<td>0.989</td>
<td>0.995</td>
<td>-0.064</td>
<td>3.92</td>
</tr>
</tbody>
</table>

* Regression equation: y = mx + c.

Blood. Other samples are plasma.

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