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**Chlorzoxazone Mimics Aprobarbital In Toxi-Lab Drug Screen**

*To the Editor:*

Recently a drug screen was request-
ed for a patient admitted to the emer-
gency room, a suspected drug-overdose victim. We used the Toxi-Lab Screen* (Marion Laboratories, Kansas City, MO 64114) of urine (Toxi-A) and se-
rum (Toxi-B). The patient was suspect-
ed of having ingested a combination of Tylenol no. 3 (acetaminophen plus code-
deine), metronidazole, and ethanol. Re-
sults of the Toxi-A screen were consist-
ent with the presence of acetamino-
phin, nicotine, and an unidentified component with an *Rf* slightly greater than that of acetaminophen. The Toxi-
B screen was consistent with the pres-
ence of aaprobarbital. We quantified the acetaminophen by liquid chromatog-
raphy, but an EMT$_{R}$ assay, run to con-
firm the presence of a barbiturate, was nega-
for barbiturates.

Emergency room personnel suggest-
ed the possibility of the presence of "Parafon forte," a fairly frequently pre-
scribed formulation of chlorzoxazone and acetaminophen. A sample of Para-
fon- forte was obtained, and it and the patient's serum were developed on du-
licate Toxi-A and B chromatograms. The Toxi-A chromatograph showed, in ad-
dition to acetaminophen, the pres-
ence of a component with an *Rf* of 0.8, which gave a pinkish-red color in stage one and did not appear in subsequent stages. The Toxi-B chromatogram showed a component co-migrating with aprobarbital. The duplicate chromato-
grams were not stained; instead, the sections with the *Rf* corresponding to the suspect chlorzoxazone were cut out and eluted with chlo-
form. The chloro-
form extract was isolated and evapo-
rated, the residue was dissolved in an
appropriate solvent, and the samples
were chromatographed on a C$_{18}$-re-
versed-phase liquid-chromatographic
column. Comparison with a chlorzoxa-
zone standard confirmed the identity of
the component.

While this occurrence of one drug's
mimicking another in the Toxi-Lab sys-
tem may not be unique, it does show
the importance of combining the
screening ability of this system (or of
any thin-layer chromatographic sys-
tem) with a confirmation test, when
possible, to reduce the clinical impact
of erroneously reporting the presence
of a particular drug.

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**Measurement of Sex Hormone
Binding Globulin**

*To the Editor:*

There are several clinical situations
in which, because of altered concentra-
tions of sex hormone binding globulin (SHBG), assay of total testosterone
provides a poor index of testosterone
status. However, direct measurement
of free testosterone is time consuming
and unsuitable for routine use, al-
though it may be calculated by using
the mass-action equations if the con-
centrations of both testosterone and
SHBG are known. (Changes of albu-
min concentration theoretically alter
the calculation, but the effect is small.)

Unfortunately, measurement of
SHBG as dihydrotestosterone (DHT)
binding capacity is cumbersome. We
have, however, previously shown that
results obtained by using the relatively
simple technique of radial immunodif-
fusion (RID) for the assay of SHBG correlate well with those obtained by
the DHT-binding method.

We have now compared RID, using
antisera and standards supplied by
Behringwerke, Marburg/Lahn, F.R.G.,
with a new noncompetitive liquid-
phase immunoradiometric assay (IRMA)
for SHBG, using kits supplied by
Farmos Diagnostica, Oulunsalo, Fin-
land. Measurements by both methods
were made on 151 samples from nor-
mal subjects and patients with liver
disease, benign prostatic hypertrophy,
carcinoma of the prostate, and hirsut-
ism. Some of these patients were being
-treated with estrogen, and their SHBG values ranged from 18 to 650 nmol/L.

An example of the relationship be-
tween the results obtained by the two
methods is shown in Figure 1 for
normal subjects over 50 years old
(19 men, 20 women). A similar rela-
tionship was found in all the patient
groups examined, and the overall cor-
relation coefficient was 0.97.

Regression analysis by use of Dem-
ing's method (4) gave *y* = 6.7x − 13
where *y* was IRMA (nmol/L) and *x* was
RID (mg/L). The residual variance
about the regression line, expressed as
a coefficient of variation (CV), was
16.5% in either direction. Because each
method has an analytical CV of about
10%, all discrepancies between the
methods can be accounted for by im-
precision, i.e., the two methods are mea-
suring the same protein in each sample.

Evidently both methods (RID and
IRMA) will provide the basis for routine
SHBG analyses. However, if the funda-
mental datum required is testosterone
binding capacity it may be appropriate
to calibrate against a DHT-binding
method. The RID has the drawbacks of
door sensitivity and the skillful prepa-
ration of the plates required for con-
sistent results, vs the advantages of
low cost. SHBG is stable in stored se-
rum, so batch running can make the
assays more cost effective.

Although measurement of free tes-
ostosterone has theoretical advantages
over measurement of total testosterone,
it is still uncertain whether the extra
information will be of clinical use, even in those situations (e.g., pa-
ents receiving estrogen therapy) when values for total testosterone can be quite misleading. However, the immu-
unological assays now available will
allow the role of free testosterone mea-
surements to be assessed.

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Analytical Performance of the Ion-Selective Electrodes Used in the Hitachi 705

To the Editor:

For determinations of Na⁺, K⁺, and Cl⁻ in clinical chemistry, ion-selective electrodes (ISE) are progressively replacing the traditional systems (e.g., flame photometry, coulometry). Recently Fogh-Andersen et al. described two ISE systems (Clin Chim Acta 30: 433–436, 1984). We have been using such a system on the Hitachi 705 discrete analyzer (Naka Works, Katsuda, Japan) for one year, and wish to report our experience with this system.

In the beginning we encountered several problems, mainly owing to a misunderstanding of the ISE system and the change from a traditional method to a new one. We believe the following requirements are necessary for optimal utilization of the technique:

- high-quality water, which in our laboratory is supplied by a system involving inverse osmosis.
- a simple, though rigorous, maintenance scheme, ensuring the reliability of the results.
- a "homemade" serum pool rather than a commercial serum for daily standardization; the accuracy of the results depends highly on the quality of this standard serum.

Under these optimal working conditions, we obtained the following results:

The correlation with results by flame photometry and coulometry was excellent. Based on 100 observations, the correlation coefficient was 0.98 for Cl⁻, 0.96 for Na⁺, and 0.99 for K⁺. For Na⁺ and K⁺, we obtained comparable results with the flame photometer, with Li or Cs internal standard.

In a further study of accuracy, we measured the three ions in an extensively dialyzed serum pool (therefore with no Na⁺, K⁺, and Cl⁻) that we had supplemented with weighted quantities of NaCl or KCl. The protein concentrations remained constant in each sample. The linearity of the specific electrode reaction to ion concentration extended from 20 to 240 mmol/L for Na⁺, 1 to 12 mmol/L for K⁺, and 70 to 130 mmol/L for Cl⁻.

The reproducibility of results for three different ion concentrations varied by less than 0.5% (CV) for each electrolyte (Table 1). In no case did the within-day or day-to-day CV exceed 0.5%.

Figure 1 shows the value of the compensation coefficient over a 50-day period. This coefficient was calculated during the standardization procedure to compensate for the effect of the proteins present. Assessing the stability of this factor as well as the daily graphical report of the values allowed us to control the stability of standardization at the beginning of each day. This compensation coefficient amounted to 2% for Na⁺, 3% for K⁺, and 5% for Cl⁻. In each case, the coefficient increased as the electrode aged.

We conclude that with our optimal working conditions the electrolyte system on the Hitachi 705 is analytically reliable. It requires a strict, yet simple, maintenance and has the advantage of needing only one daily standardization. The addition of a bacteriostatic agent to the reagent improves maintenance and reduces contamination by pyocyanic bacilli, to which the K⁺ electrode is particularly sensitive.

One shortcoming, however, is that this system does not allow ion determinations on biological media such as hemodialysis liquids, cerebrospinal fluid, or urine.

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Better Quality Control of Serum CK-MB Determinations

To the Editor:

Detection of the MB isoenzyme (CK-MB) of creatine kinase (EC 2.7.3.2) in serum is useful in the diagnosis of acute myocardial infarction. Increased (or an increased proportion of) CK-MB activity in the serum is evidence in favor of a diagnosis of myocardial ischemia or necrosis. Decision values for CK-MB, based on results of clinical studies, are used to distinguish between individuals with and without acute myocardial infarction. Patients with acute myocardial infarction reportedly will have a CK-MB value of >3% (1) or >5% (2). These values are consistent with the manufacturer’s claim for the Corning agarose electrophoresis system (Corning Medical and Scientific, Medfield, MA 02052), a widely used procedure. According to Corning’s data (3), if 3% is used as the CK-MB cutoff value, the diagnostic sensitivity and specificity are 96.4% and 37.3%, respectively; if 5% is used, these values are 75.1% and 100%, respectively. The total CK reference range in the Corning study was 15 to 165 U/L. The analytical sensitivity (detection limit) of the procedure for CK-MB is 2 U/L.

Performing the CK-MB assay in our laboratory with the Corning agarose electrophoretic procedure, we noted that our quality-control material was not monitoring the performance of the electrophoretic procedure at the decision level, a defect shared by various other quality-control products, e.g., CK isoenzyme-control products from Beckman, Corning, Dade, Fisher, Helena, Ortho, and Roche. Except for the Level I Corning control, all other materials surveyed had high activities of CK-