Morphine was determined by the method of Ratcliff and Hardwicke (6).

Serum haptoglobin concentration in the normal controls was 1439 ± 405 mg/liter; in the cases of pure iron deficiency it was 796 ± 323 mg/liter. In the cord sera the concentration of haptoglobin was 195 ± 112 mg/liter. The patients' values for serum haptoglobin were clearly (P < 0.001) lower than those of normal controls.

It is difficult to find cases of pure iron-deficiency anemia in our population. Usually other nutritional deficiencies are associated. Thus the number of cases we studied is small. Still, a highly significant decrease in serum haptoglobin was seen in these patients. This decrease may be a sign of mild hemolysis in these cases, equivalent to 640 mg of hemoglobin per liter; that is to say, rather less than 1% of the total amount of hemoglobin in the blood.

References

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Formaldehyde Interference with Fluorescence Determination of Morphone in Urine

To the Editor:

While evaluating a routine confirmatory fluorescence procedure (1) for morphone, we discovered an interference that resulted in falsely negative results for morphone. Five samples, consisting of morphone-free human urine and human urine supplemented with morphone sulfate in concentrations ranging from 500 to 1000 µg/liter, were submitted by a state health department. On analysis by thin-layer chromatography (2), three urines were positive for morphone and two were negative. However, confirmatory analysis by the morphone fluorescence procedure gave negative results for all five samples. The fluorescence procedure was checked for possible problems with a blind sample—run of positive and negative morphone urine proficiency-testing samples from the Center for Disease Control (CDC). Results from these CDC samples correlated correctly with morphone supplementation.

We learned that the state laboratory had used small amounts of 37% formaldehyde solution (from 10 ml per 5 liters of urine to 5 ml per 16 liters of urine) as a urine preservative. To determine whether formaldehyde was responsible for the false-negative results, we used two sets of 10 CDC proficiency-testing samples in conjunction with a duplicate set of the samples from the state health department. To one set of CDC samples was added formaldehyde solution (0.07 ml per 35 ml of urine); a control set was left untreated. Thin-layer chromatographic analysis of all the samples confirmed the known presence or absence of morphone. However, the results by the fluorescence procedure disagreed. All the fluorescence scans of the state's samples and of the CDC formaldehyde-supplemented samples, which included a total of seven morphone-containing samples, were negative for morphone, whereas the control samples containing no formaldehyde gave four true positive and six true negative results. As a test of whether or not the interfering substances could be removed by the chromatography, aliquots from the same samples were separated by thin-layer chromatography, visualized, scraped, re-extracted, and analyzed by fluorescence. The fluorescence scans of the formaldehyde-supplemented samples, the unsupplemented control samples, and the state samples correlated correctly with their results by thin-layer chromatography. Since the only difference in the two CDC sample sets was the addition of formaldehyde, and since the results mimicked the problem with the state samples, we concluded that formaldehyde was interfering.

Formaldehyde interference may not be relevant in most clinical situations; however, proficiency-testing samples, in-house control pools, and postmortem specimens might contain formaldehyde, which could subsequently interfere with the analysis for morphone by a fluorescence procedure.

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"Disappearing" Lipoprotein-X

To the Editor:

A recent issue of Clinical Chemistry (23, 2302 (1977)) contains an excellent article by Mucke and coworkers who describe the "disappearance" of lipoprotein-X (LP-X) after in vitro addition of oleic acid. We agree with the statement of Dr. Muckle that this is not due to LP-X destruction, but rather to changes on electrophoretic migration characteristics, as we have shown (1). Furthermore, mechanisms other than fatty acids are also involved in postheparin electrophoretic behavior changes. We wish to comment on several points:

(a) The enzyme released after heparin treatment that induces LP-X electrophoretic changes seems to be a phospholipase, as we have shown recently (2), although free fatty acids released from parallel plasma lecithin transformation could play an important role, as Muckle and coworkers suggest.

(b) The fact that "disappearance" of LP-X is due to a change in the electrophoretic characteristics has been suggested by Ritland et al. (3), because serum LP-X postheparin incubation induced no changes evident on electron microscopic examination. Lately, in agreement with suggestions of Ritland and experiments of Muckle et al., we have demonstrated (1) that when [14C]cholesterol-LP-X, isolated from the cathode by agar gel electrophoresis, was incubated with phospholipase A and also with deoxycholic acid, LP-X was found on the anodal side by visual examination and by measuring the labeled cholesterol on a second agar gel electrophoresis procedure.

(c) All these observations on falsely negative LP-X tests induced by several substances—such as phospholipase,
oleic acid, deoxycholic acid, and probably many others—could help explain the negative LP-X results in some patients with cholestasis. Seidel et al. (4) suggested that bile salts could explain in part the negative LP-X in long-lasting cholestasis. To investigate this hypothesis we have studied (to be published) the relationship between serum LP-X and serum bile salt concentrations in patients with cholestasis; neither a positive or a negative correlation was found. Furthermore, the concentrations of different bile salts required in vitro to induce negative LP-X are up to about 2000 μmol/litre, more than fivefold the concentration of serum bile salts usually found in patients with severe choles-

tasis.

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Aspartate Aminotransferase
Activity of Some Commercially
Available Control Sera, Measured
with and without Added Pyridoxal-
5-Phosphate

To the Editor:
The diagnostic importance of aspartate aminotransferase (EC 2.6.1.1) measure-
ments in clinical medicine is well established. It is also well known that the aminotransferase requires pyridoxal-5-
phosphate (Pyr) for its catalytic ac-
tivity (1, 2). Several recent reports in-
dicate that the aminotransferase in serum may not be fully saturated with Pyr and catalytic activity can be enhanced after

dition of Pyr P in vitro (3-5). Fur-
thermore, the degree of reactivation of the aminotransferase varies markedly in nor-

mal individuals and in different disease processes (3). In the provisional recom-

mendations for the measurement of this enzyme, the expert panel of the Inter-

ational Federation of Clinical Chemistry (IFCC) includes Pyr P in the reaction mixture (6). If these recom-

mendations are accepted, it will ob-
viously be necessary to establish new reference ranges and for manufacturers of quality-control sera to assign new

values to their products based on the recom-

mended procedure. Preliminary obser-
vations in our laboratory demonstra-
ted that the catalytic activity of this enzyme in different commercial quality-

control products was indeed mark-
edly affected by the addition of Pyr P to the reaction mixture (Table 1).

When the reaction mixture was sup-
plemented with Pyr P, much higher values were obtained for Precipath E (Boehringer), where the degree of reac-
tivation was only 5%. The values given in Table 1 were obtained 1 h after re-
constitution of the lyophilized sera. No change in the degree of reactivation of the aminotransferase occurred in the samples during a period of 24 h after the initial measurements were made.

To avoid confusion, it is therefore essen-
tial that manufacturers of quality

control sera should in future supply the values for aspartate aminotransferase activity with and without added Pyr P.

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Table 1. Aspartate Aminotransferase Activity of Some Commercially
Available Control Sera without and with Pyr P Supplementation

<table>
<thead>
<tr>
<th>Serum</th>
<th>n</th>
<th>Pyr P (U/liter)</th>
<th>Pyr P (U/liter)</th>
<th>Stimulation, %</th>
</tr>
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<tbody>
<tr>
<td>Precipath E</td>
<td>5</td>
<td>153</td>
<td>160</td>
<td>5</td>
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<tr>
<td>(Boehringer)</td>
<td>20</td>
<td>(149–155)</td>
<td>(158–165)</td>
<td>(3–7)</td>
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<tr>
<td>Quality Assurance</td>
<td>6</td>
<td>70</td>
<td>103</td>
<td>47</td>
</tr>
<tr>
<td>Serum</td>
<td>6</td>
<td>(67–73)</td>
<td>(96–107)</td>
<td>(45–52)</td>
</tr>
<tr>
<td>(General Diagnostics)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Versatol-E</td>
<td>3</td>
<td>218</td>
<td>480</td>
<td>120</td>
</tr>
<tr>
<td>(General Diagnostics)</td>
<td>14</td>
<td>(212–231)</td>
<td>(486–500)</td>
<td>(118–125)</td>
</tr>
<tr>
<td>Wellcomrol One</td>
<td>3</td>
<td>58</td>
<td>106</td>
<td>83</td>
</tr>
<tr>
<td>(Wellcore)</td>
<td>7</td>
<td>(56–59)</td>
<td>(105–107)</td>
<td>(79–84)</td>
</tr>
<tr>
<td>Normal Enzyme</td>
<td>3</td>
<td>13</td>
<td>29</td>
<td>123</td>
</tr>
<tr>
<td>Control Serum</td>
<td>3</td>
<td>(12–13)</td>
<td>(28–29)</td>
<td>(118–125)</td>
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<tr>
<td>(Ortho Diagnostics)</td>
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</table>

* n, no. samples.

Two Procedures Compared for Determination of Plasma
Cholinesterase Activity

To the Editor:
We have compared the bmc choline-
sterase (EC 3.1.1.8) reagent set (Bio-
dynamics/bmc, Indianapolis, Ind. 46250) modification for the Gilford 3500
Series Chemical Analyzer (Gilford In-
strument Laboratories, Inc., Oberlin,
Ohio 44074) with the Michel (1) poten-
tiometric procedure. The bmc procedure is photometric and based upon the work of Ellman et al. (2); it can be run at the rate of 77 determinations per hour, the Michel procedure at about 20 to 30 per hour. The Michel method, done manu-

ally, is subject to several potential sources of error, both human and

chemical, such as pH of the buffer-

substrate, temperature, reaction time,