whole-blood hemolysate, it was found to represent 3.8% false glycohemoglobin.

Interference from lactescence can be decreased by measuring the difference in absorbance between 415 and 450 nm, as is done automatically by the ABA-100 (Abbott Laboratories, North Chicago, IL 60664). When the above experiment was measured on the ABA-100, lactescence in the plasma was found to represent only 0.9% false glycohemoglobin.

Glucose intolerance has been strongly linked to types IV and V hyperlipoproteinemia (2), and lactescing plasma is common in these patients (2). Lactescence would be particularly severe postprandially, and fasting is not normally required in glycohemoglobin analysis. Thus measuring falsely high glycohemoglobin is a practical risk in these patients and would further complicate the interrelationships of glucose intolerance, diabetes, and hyperlipoproteinemia.

References

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Carbamazepine and Urinary 17-Hydroxycorticosteroids

To the Editor:

Carbamazepine (Tegretol®) should be added to the list of drugs that can cause a false increase in the values for apparent 17-hydroxycorticosteroids (17-OHCS) in the urine as measured by the modified method of Reddy–Jenkins–Thorn (1).

Two women receiving carbamazepine, 600 mg daily for four weeks, for the treatment of trigeminal neuralgia, were found to have increased urinary 17-OHCS, while values for 17-ketosteroids (17-KS) were within the normal range. Measurements after discontinuation of the drug gave normal values for both (Table 1).

Arise et al. (2) investigated the mechanisms of interference of carbamazepine with the Porter–Silber reaction after acute administration of 400 mg of the drug to two subjects for one day. They concluded that it is due to the formation of acridine 9-aldehyde phenylhydrazone as one of the drug metabolites.

To study further the acute effect of the drug, we gave seven healthy volunteers carbamazepine in doses of up to 600 mg/day for one or two days. Twenty-four-hour urine 17-OHCS showed no significant change during or on the two days after this drug administration. Thus it appears that interference of the drug with 17-OHCS determination is either seen in only some subjects or seen mainly after prolonged administration of the drug. A further study in patients on long-term treatment with carbamazepine is recommended.

References

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<table>
<thead>
<tr>
<th>Table 1. Urinary 17-OHCS, 17-KS, and Creatinine of Two Patients during and after Administration of Carbamazepine</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Dose of carbamazepine (mg/day)</strong></td>
</tr>
<tr>
<td>-----------------------------------</td>
</tr>
<tr>
<td>Urinary 17-OHCS (mg/24 h)</td>
</tr>
<tr>
<td>Urinary 17-KS (mg/24 h)</td>
</tr>
<tr>
<td>Urinary creatinine (g/24 h)</td>
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<td>17-OHCS/g creatinine</td>
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Table 1. Formation of the Zn-Zincin Complex in Various Buffers

<table>
<thead>
<tr>
<th>Buffer</th>
<th>$pK_a$</th>
<th>$pH$</th>
<th>$\Delta A_{520}$</th>
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<tr>
<td>(NaOH)</td>
<td>—</td>
<td>10.2</td>
<td>0.879</td>
</tr>
<tr>
<td>2-Amino-2-methyl-1-propanol</td>
<td>9.7</td>
<td>10.2</td>
<td>0.814</td>
</tr>
<tr>
<td>2-Amino-2-methyl-1-propanol</td>
<td>9.13</td>
<td>0.876</td>
<td></td>
</tr>
<tr>
<td>2-Amino-2-methyl-1-propanol</td>
<td>7.40</td>
<td>0.832</td>
<td></td>
</tr>
<tr>
<td>Borate</td>
<td>9.2</td>
<td>10.2</td>
<td>0.814</td>
</tr>
<tr>
<td>Borate</td>
<td>9.10</td>
<td>0.783</td>
<td></td>
</tr>
<tr>
<td>Borate</td>
<td>7.42</td>
<td>0.772</td>
<td></td>
</tr>
<tr>
<td>HEPES</td>
<td>7.55</td>
<td>7.40</td>
<td>0.723</td>
</tr>
<tr>
<td>MES</td>
<td>6.15</td>
<td>7.42</td>
<td>0.708</td>
</tr>
<tr>
<td>Tris</td>
<td>8.1</td>
<td>7.43</td>
<td>0.585</td>
</tr>
<tr>
<td>TES</td>
<td>7.5</td>
<td>7.41</td>
<td>0.397</td>
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<tr>
<td>Triethanolamine</td>
<td>9.5</td>
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<td>0.243</td>
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<td>Phosphate</td>
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<td>EDTA</td>
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</table>

Table 1. Binding of Zn²⁺ by Buffers

To the Editor:

Recently Williamson and Thompson (1) reported that the buffer 2-amino-2-methyl-1-propanol may contain an impurity that inhibits alkaline phosphatase (EC 3.1.3.1) activity at pH 10.15. They suggested that the impurity may be an ethylendiamine derivative that binds Zn²⁺.

In another connection, I have estimated the binding of Zn²⁺ by a number of common buffers at pH 7.4 (unpublished) and now include measurements with 2-amino-2-methyl-1-propanol. The indicator 2-carboxy-2'-hydroxy-5'-sulfoformylbenzene (zincin) can be conveniently used for this purpose. Rush and Yoe (2) described the determination of Zn²⁺ with this indicator in borate buffer at pH 9.0. At this pH, free zinc is orange ($\lambda_{max}$ 488 nm, $\epsilon_{mmol}$ about 27) and the Zn-zincin complex is blue ($\lambda_{max}$ 620 nm, $\epsilon_{mmol}$ about 23). The absorbance change at 620 nm is linear with Zn²⁺ concentration and the dye can be titrated photometrically at pH 9 and 620 nm with a standard Zn solution.

The buffer compounds were dissolved in distilled de-ionized water, adjusted to the desired pH, and diluted to 0.10 mol/L. To 3.00 mL of each buffer, in cells of 10-mm light-path, were added 50 µL of zinc solution, 3.32 mmol/L, and then 50 µL of standard ZnSO₄, 1.80 mmol/L. I noted the increase in absorbance at 620 nm, which is a measure of the free Zn²⁺, that is, the Zn²⁺ not bound by the buffer. The results are given in Table 1, with the buffers listed in order of increasing Zn²⁺ binding.

It will be seen that 2-amino-2-methyl-1-propanol binds less Zn²⁺ than does borate, and EDTA binds the cation completely. In a spectrophotometric titration of the dye at 620 nm, the amimonethylpropanol gave a sharper end-point than did borate. Hanlon et al. (3) used a potentiometric titration method to show that Tris binds Zn²⁺. It is concluded that this particular sample of 2-amino-2-methyl-1-propanol binds no appreciable amount of Zn²⁺. The buffers that do bind appreciably

CLINICAL CHEMISTRY Vol 25 No 3 1979 495
should not be used with enzyme systems that require Zn\(^{2+}\).

Zinc and the standard ZnSO\(_4\) solution were from J.T. Baker; Tris (THAM) and sodium tetraborate were Fisher Certified. The 2-amino-2-methyl-1-propanol was from Sigma (pH lot No. 14C-3610) as were the Good's buffers 4-morpholineethanesulfonic acid (MES) and 2-[(2-hydroxy-1,1-bis(hydroxymethyl)ethyl]aminoethanesulfonic acid (TES). The other Good's buffers, N,N-bis(2-hydroxyethyl)glycine (Bicine), 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), and N-[2-hydroxy-1,1-bis(hydroxymethyl)ethyl]glycine (TRICINE), were products of Calbiochem. The disodium EDTA was British Drug Houses AnalR grade, and triethanolamine (2,2'2' nitrotriethanol-HCl) was from Eastman Organic Chemicals.

References

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Evaluation of the Creatine Kinase MB Kit of Boehringer

To the Editor:

The original method of Mercer (1) for separation of isoenzymes of CK (creatinine kinase, EC 2.7.3.2.,) when slightly adapted, becomes a practical method in daily laboratory routine (2). The CK-MB kit of Boehringer (no. 189219), which became recently available, is based on ion-exchange chromatography on minicolumns. An evaluation of the Boehringer column method compared with the Mercer method has been reported recently (3). In this communication, results obtained with the Boehringer CK-MB kit are compared with those obtained with the Mercer technique as slightly modified (2).

CK-isoenzyme separation with the kit was performed as described in the instruction sheet accompanying the kit (cf. 3). CK-isoenzyme separation according to Fiolet et al. was performed as described previously (2), except that an extra separate 2 mL of primary elution buffer was included to ensure that carryover of CK-MM isoenzyme in the CK-MB fraction was less than 0.2% at total CK activities greater than 2000 U/L.

Sample handling and assay conditions of CK-MB eluates obtained with both methods were as described previously (2).

Plasma samples were obtained from patients with or without myocardial infarction, presented or admitted to the coronary care unit.

For the study of carryover of CK-MM, a CK-MB-free pooled plasma was used, supplemented with semi-purified human CK-MM; total CK-MM activities ranged from 2000 to 5000 U/L.

The regression of the results obtained with the Boehringer kit against those with the Mercer method modified according to Fiolet et al. (2) shows satisfactory correlation (r = 0.998, n = 38); also, the actual values found agree rather well (slope = 1.03, y-intercept = -0.2). Activities of CK-MB were determined over the whole range of values routinely observed in patients with myocardial infarction (480 U/L). Also in the range of very low CK-MB activities (<4 U/L) the measured values were in good agreement: for the Boehringer kit, 1.9 ± 0.9 U/L (mean ± SD, n = 20); for the method of Fiolet et al., 1.8 ± 1.0 U/L (mean ± SD, n = 20).

The percentage of total CK that was CK-MB with the method of Fiolet et al. was found to be 10 to 12% if the blood sample was drawn in the rising part of the CK-release curve, if the infantart was not too small, and if extra-cardiac CK release was absent (cf. 2).

An estimate of day-to-day precision with the kit was obtained with the CK-MB control serum included in the kit: 38.9 ± 2.6 U/L (mean ± SD, n = 22). The observed CV of 6.7% is somewhat larger than the value of 4.3% stated in the instruction sheet, but is comparable to that found with the method of Fiolet et al. (cf. 2, 3). The observed deviation of the mean value found for the control from the stated value of 51 U/L is due to differences in the assay conditions; glutathione was used as enzyme activator instead of N-acetylcycteine, and the sample volume fraction was different from that prescribed in the instruction sheet of the kit. Correction of the observed mean value of 38.9 U/L, allowing for these differences, gives a corrected mean value of 47 U/L for the control serum.

Carryover of CK-MM into the CK-MB eluate never exceeded 0.17% with pooled sera enriched with human CK-MM up to activities of 5000 U/L and averaged 0.1% (n = 16), considerably less than the 1% mentioned in the instruction manual.

The complete separation procedure with the Boehringer CK-MB kit requires about 25 min for a single sample, much faster than the comparison method (about 45 min). However, with larger series of samples to be analyzed (about 20) the time required for separation becomes comparable. In this case the kit compares unfavorably with the comparison method because it is more laborious.

The previous evaluation of the BMC column method by McQueen et al. (3) agrees with our results in that the methods compared are linearly related with a high correlation coefficient, carryover of CK-MM is low, reproducibility is satisfactory, and the percentage of CK-MB found is comparable.

However, it is not well understood why the slope of the regression line found by McQueen et al.(3) is only 0.679, whereas in the present report the slope is 1.02, especially in view of the fact that the method of Fiolet et al. is not fundamentally different from that of Mercer.

We concluded that the Boehringer kit provides a reliable method of CK-MB separation and seems especially useful for on-site determinations and for small series of plasma samples.

References

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Hyperglycemia-induced Hyponatremia: A Fresh Look

To the Editor:

It has long been recognized that hyperglycemia depresses serum sodium concentration (1), and several investigators have examined the mechanism and extent of this depression. Seldin and Taraili (2) considered that administered hypertonic glucose is largely confined to the extracellular fluid (ECF), thereby producing osmotic extraction of water from the intracellular fluid (ICF) and resulting hyponatremia. If sufficient