Both CPK and LDH determinations may give erroneously high results in such a system, especially if a computer printout is used.

Boehringer Mannheim has investigated the possibilities of shortening the "lag-phase" for CPK determinations. It was found that the use of chromatographically pure creatine phosphate in the assay reduces the phase to less than 1 minute, but costs increase four to five times. To rectify the problem for routine laboratory, the following steps have been taken:

1. Since July 1971, Canadian customers who use the CPK kit (BMC Cat. No. 15926) with the LKB 8600 are given ATP-free creatine phosphate free of charge.

2. During 1972, a new CPK test kit (BMC Cat. No. 15721) was introduced, especially designed for the use with the LKB 8600 and other automated instruments. This kit contains ATP-free creatine phosphate of the highest purity.

We must emphasize that our Test Combination (BMC Cat. No. 15926) for the determination of CPK activity does give correct results when used according to the printed insert. The methodology is based on proposals made by Rosalki. Any modification to the operating instructions should first be discussed with our Technical Services Department.

It is regretful that often attempts to economize the work-load results in over-asking for the possibilities of the given instrument-reagent combinations.

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Boehringer Mannheim Corporation was asked to respond to the preceding letter, and offers the following reply:

To the Editor:
The LKB 8600 Reaction Rate Analyzer sold in the United States, is equipped with a "delay switch," whereas the same instrument sold in Canada measures time rate reactions immediately after the addition of the initiating reagent, without provision for delay. Therefore, "lag-phases" of any enzyme reaction are recorded with the Canadian version. One of the basics of zero-order kinetic assays is the measurement of the linear phase of reaction. This rule cannot be observed if an instrument such as the LKB 8600 is used without a modification to permit delayed measurements.

The percent of expected value for the above data was thus calculated:

\[
\text{Me} = \frac{[3.5/2.2 + 2.5]}{100} \times 74\% \\
\text{En} - \text{FH}_4 \times \% \\
\text{dog} - \text{added Ex} - \text{Folate expected as - pected folate serum folate sayed value}
\]

2.2 2.5 4.7 3.5 74

The accepted method of evaluating an analytical test is by means of the percent recovery, which is calculated by dividing the amount recovered ("folate assayed" minus the "endogenous folate") by the amount of methyl-FH4 added, and multiplying by 100.

Applying this method to the same data given above yields the following result:

\[
\% \text{ recovery} = \left(\frac{3.5 - 2.2}{2.5}\right) \times 100 = 52\%
\]

A comparison of the percent expected value and percent recovery (calculated by me) for the data of Rothenberg et al. is given in Table 1.

The mean percent recovery for the data in Table 1 is 93% ± 40.1 (mean ± standard deviation), with a range from 20% to 160%. The coefficient of variation (in percent) is the ratio of the standard deviation to the mean multiplied by 100, and is widely used.
in clinical chemistry to express the relative precision of an analytical procedure. The coefficient of variation for the percent recoveries is 44, indicating that the precision for their recoveries is inadequate. The imprecision of the recovery data is not evident to the reader who assumes that percent of expected value is equivalent to the percent recovery. The difference in these methods is evident when the data in the Table are examined. For example, the percent recovery for sample number 11 is only 37%; but the percent of expected value is 78%.

Third, Rothenberg et al. discount Waxman et al.'s explanation (3) for substantial differences in values between the latter group's radioassay procedure and L. casei bioassay for serum folates. Waxman et al. attributed these differences to using a racemic mixture of methyl-FH4 as their standard. They assumed that only one of the isomers binds to the milk protein. The data of Rothenberg et al. seem to indicate that an excess of milk binder will bind both isomers. However, the experiments of Rothenberg et al. cannot be interpreted as demonstrating that both isomers have the same affinity for the folate-binding protein. Additional competition experiments with the separated enantiomers of methyl-FH4 are required to determine which isomer has the higher affinity for the milk folate-binding protein and whether the discrepancy between L. casei and radioassay analysis is due to the use of the racemic mixture of methyl-FH4 as a standard.

Clearly, much additional work is needed before this radioassay for serum folic acid can replace the conventional L. casei assay for general use.

References

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Editor's note: With regard to the recovery question, this same point was recently made elsewhere [Amer. J. Med. Technol. 38, 274 (1972)] by Plaut et al. Evidently there is widespread misconception of this convention.

<table>
<thead>
<tr>
<th>Case I</th>
<th>True value, g/dl</th>
<th>Experimental value (max. 5% error)</th>
<th>Relative error in globulin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tot. protein</td>
<td>7.50</td>
<td>7.88</td>
<td>[3.6 - 3.0]/3.00</td>
</tr>
<tr>
<td>Albumin</td>
<td>4.50</td>
<td>4.28</td>
<td>[7.50 - 7.13]/7.50</td>
</tr>
<tr>
<td>Globulin (by difference)</td>
<td>3.00</td>
<td>(3.60)</td>
<td>$100 = 20%$</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Case II</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Albumin</td>
<td>4.50</td>
<td>4.28</td>
<td></td>
</tr>
<tr>
<td>Globulin</td>
<td>3.00</td>
<td>2.85</td>
<td></td>
</tr>
<tr>
<td>Tot. protein</td>
<td>7.50</td>
<td>(7.13)</td>
<td></td>
</tr>
</tbody>
</table>

### Serum Globulin Assay

To the Editor:

We call attention to the relatively large errors that can occur when serum globulin is assayed by difference between total protein and albumin, as is common practice today.

The following assumptions are made:

(a) A maximum 5% error in each assay.

(b) The errors for each assay are in opposite directions in Case I and in the same direction in Case II.

(c) The true values for total protein and albumin are 7.5 and 4.5 g/dl. This gives 3.0 g/dl as the true value for globulin by difference. Data for the above values are tabulated below. Also tabulated are the results obtained by assaying albumin and globulin independently and calculating total protein from their sum.

The above data point to the great need for carrying out globulin assays independently and not by difference calculations.

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### Enzyme Determinations: Criticisms of Some Recent Reports

To the Editor:

We are troubled by the appearance, in recent issues of Clinical Chemistry, of two reports that offer false and unexamined assumptions as if they were facts.

In a recent note, Wolf et al. (1) have suggested that low aspartate aminotransferase (AST; EC 2.6.1.1.) activity might be due to "high serum lactate concentrations, which caused a rapid consumption of NADH coenzyme in the chemical laboratory test." NADH oxidation by lactate is a reaction unfamiliar to us, as is the reduction of lactate to pyruvate, reported by these authors. These unusual chemical reactions were not referenced by the authors. In addition, the reference that they cite for artifactually low AST activities and (or) high serum lactate concentrations is a paper that discusses neither (2). On the contrary, that paper describes elevated serum AST activities in two of the three conditions cited by Wolf et al.

We have examined the effects of lactate on the malate dehydrogenase (MD; EC 1.1.1.37) coupled assay for AST. Since decrease in NADH concentration is measured, lactate-mediated consumption of NADH during activity measurements would of course give rise to artifactually enhanced aminotransferase activities. We therefore examined the effect of lactate on NADH during preincubation. We were unable, however, to show any influence by lactate, up to final concentrations of 62 mmol/liter, on the preincubation NADH concentration in the kinetic AST method. A decrease in NADH absorbance is observed when lactate contains dinitrophenyl-hydrazine reactive materials—a contamination we have frequently encountered. (These data, which suggest that commercial lactic acid is often contaminated by pyruvate, should be investigated by users of lactate-to-pyruvate estimations of serum lactate dehydrogenase activity.)

However, it is conceivable that high serum lactate concentrations might lead to artifactually low AST activities by a totally different scheme, the recycling of coenzyme, catalyzed by serum lactate dehydrogenase (LD; EC 1.1.1.27) (Figure 1). We therefore evaluated the effect of lactate on the kinetic estimation of AST activity. L-Lactic acid, 40% solution, was purchased from Schwartz Mann, Orangeburg, N.Y., and aminotransferase activity was assayed at 30°C under conditions we have previously described (3). The sample