Letters to the Editor

A Source of Error in Blood Pyruvate Determinations

Blood pyruvate determinations can readily be carried out using the convenient enzyme kits marketed by many commercial firms. While using one of these kits I have observed a source of error when measuring recoveries of standard pyruvate solutions added to either trichloroacetic acid-HCl extracts (Gloster and Harris, 1962) or perchloric acid extracts (Landon, Fawcett, and Wynn, 1962). This error only becomes apparent when the added standard is about four times the usual resting venous blood levels but it nevertheless could become important when high levels of pyruvate are being measured in blood.

The accompanying Table shows the effect of adding ATP, phosphoenolpyruvate (PEP), or EDTA to TCA-HCl

2 Binding of these divalent ions by EDTA inhibits pyruvate kinase activity.

3 The equilibrium of the reaction, $ADP + PEP \rightleftharpoons \text{pyruvate} + ATP$ catalysed by pyruvate kinase, favours pyruvate formation but the muscle enzyme is readily reversed by the presence of ATP (Krimsky, 1959). Most lactate dehydrogenase preparations are obtained from muscle. Thus the addition of ATP to acid extracts of blood containing 200 nmol of added pyruvate causes a reduction of measurable pyruvate because of the formation of PEP.

4 The conversion of pyruvate to PEP only occurs when the levels of pyruvate are relatively high because of the (presumed) minimal contamination of lactate dehydrogenase preparations with pyruvate kinase. This reaction could be prevented by the addition of PEP to acid extracts of blood containing 200 nmol of added pyruvate. Because the levels of pyruvate in the extract are relatively low the addition of PEP alone does not produce any effect.

Most enzymatic determinations of pyruvate use buffers which do not contain

Reaction Mixture	Recovery of Added Pyruvate (%)
0.16 M Triethanolamine buffer (pH 7.5) + 200 nmol pyruvate	98
Blood extract ¹ + 200 nmol pyruvate	85
Blood extract ¹ + 200 nmol pyruvate + 600 nmol EDTA	104
Blood extract ¹ + 200 nmol pyruvate + 1 μmol ATP (5 min incubation before assay)	76
Blood extract ¹ + 200 nmol pyruvate (5 min incubation before assay)	82
Blood extract ¹ + 200 nmol pyruvate + 2.4 μmol PEP	106

Table Pyruvate recoveries from blood extracts

¹Freshly drawn blood (containing 52.2 nmol/ml endogenous pyruvate) was added to an equal volume of ice-cold 10% TCA in 0.5 mol/litre M-HCl, mixed, centrifuged and filtered at 2°C; 2 ml of the supernatant was added to 1 ml of 0.7 mol/litre K₃PO₄ (final pH 7.6) and assayed at 25°C by means of a commercially available enzymatic assay for pyruvate using a recording spectrophotometer at 340 nm.

extracts of blood containing 200 nmol of added pyruvate.

I have therefore concluded that the effect is due to slight contamination of some of the lactate dehydrogenase (EC 1.1.1.27) preparations used in blood pyruvate determinations with traces of pyruvate kinase (EC 2.7.1.40). The presumptive evidence for this conclusion is as follows:

1 Pyruvate kinase requires the presence of Na⁺ or K⁺ and Mg²⁺ or Mn²⁺ (Bergmeyer, Klotzsch, Mollering, Nölböck-Hochstetter, and Beauchamp, 1963), and ATP for the formation of PEP. These are present in the acid extract of blood but not in the 0.16 M triethanolamine buffer.

EDTA (Gloster and Harris, 1962; Landon, Fawcett, and Wynn, 1962; Hadjivassiliou and Rieder, 1968) and I would suggest that the assay system used by Hohurst, Kreutz, and Bücher (1959) containing EDTA in the buffer would overcome the occasional contamination of lactate dehydrogenase by pyruvate kinase which I have found. It is necessary to add that this contamination has been found in four kits out of 25 examined.

A. R. HENDERSON
From the University Department
of Pathological Biochemistry,
Royal Infirmary,
Glasgow

References

Bergmeyer, H. U., Klotzsch, H., Mollering, H., Nölböck-Hochstetter, M., and Beauchamp, K. (1963). Biochemical Reagents; Enzymes. In *Methods of Enzymatic Analysis*, edited by H. U. Bergmeyer, p. 997. Academic Press, New York and London.
Gloster, J. A., and Harris, P. (1962). Observations on an enzymic method for the estimation of pyruvate in blood. *Clin. chim. Acta*, 7, 206-211.
Hadjivassiliou, A. G., and Rieder, S. V. (1968). The enzymatic assay of pyruvic and lactic acids. A definitive procedure. *Clin. chim. Acta*, 19, 357-361.
Hohorst, H. J., Kreutz, F. H., and Bücher, T. (1959). Über Metabolitgehalte und Metabolit-Konzentrationen in der Leber der Ratte. *Biochem. Z.*, 332, 18-46.
Krimsky, L. (1959). Phosphorylation of pyruvate by the pyruvate kinase reaction and reversal of glycolysis in a reconstructed system. *J. biol. Chem.*, 234, 232-236.
Landon, J., Fawcett, J. K., and Wynn, V. (1962). Blood pyruvate concentration measured by a specific method in control subjects. *J. clin. Pathol.*, 15, 579-584.

Comparison of Complement Fixation and Crossover Electrophoresis for Detection of Hepatitis-associated Antigen

White, Lasheen, Baillie, and Turner (this Journal, February 1971, 8-12) claim that crossover electrophoresis is as sensitive as complement fixation for the detection of hepatitis-associated antigen (HAA). In a similar study (Pringle, McMichael, and Ross, 1971) we have found that complement fixation was at least 16 times more sensitive than crossover immunoelectrophoresis. These different results are probably due to differences in sensitivity and specificity of the detector anti-HAA sera. White *et al* used a serum from a haemophilic patient as their detector antiserum, but did not give the comparative HAA and anti-HAA titres obtained in two-dimensional titrations by complement fixation and crossover electrophoresis. Thus, the comparative sensitivity of this serum for detecting HAA by these two techniques cannot be ascertained from their paper.

In our study (Pringle *et al*, 1971) we used as detector anti-HAA pooled guinea pig serum provided by Dr L. Barker, Division of Biologic Standards, the National Institutes of Health, Bethesda, USA. In two-dimensional titrations against several human sera containing various levels of HAA the anti-HAA titre was 1 in 512 by complement fixation compared with 1 in 32 by crossover electrophoresis; at antiserum titre, HAA titres were 16-32