Letters to the Editor

A Source of Error in Blood Pyruvate Determinations

Blood pyruvate determinations can readily be carried out using the conventional 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, 1966). 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 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, Klotschz, Mollering, Nellböck-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.

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

3 The equilibrium of the reaction, ADP + PEP = 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 EDTA (Gloster and Harris, 1962; Landon, Fawcett, and Wynn, 1966; Hadjivassiliou and Rieder, 1968) and I would suggest that the assay system used by Hohorst, 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.

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References


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

White, Lasheen, Ballie, 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 antisemum titre, HAA titres were 16-32
fold higher by complement fixation than by crossover electrophoresis. To minimize the prozone in complement-fixation tests this antiserum was used in diagnostic tests for HAA at eight times titre, ie, 1 in 64; for crossover electrophoresis 1 in 16 was used. Of 107 sera from patients with hepatitis tested by both techniques, 36 were positive for HAA; all 36 were positive by complement fixation but only 31 by crossover electrophoresis. The 31 positive by both techniques had HAA titres by complement fixation ranging from 1 in 32 to 1 in 512; the five positive only by complement fixation had titres from 1 in 4 to 1 in 16. Further, we found that the prozone in complement-fixation tests was an advantage rather than a disadvantage since an approximate quantitative assessment of HAA could be obtained by screen tests on two separate dilutions (1 in 4 and 1 in 32) of each test serum. Thus, all sera showing a prozone for HAA in these two dilutions subsequently gave straight line titres \( \geq 1 \) in 64 whereas those not showing a prozone had titres \(< 1 \) in 64.

We also found that complement-fixation tests had another advantage over crossover electrophoresis in that serum dilutions of 1 in 4 could be heated at 60°C for 16 hours or 85°C for one hour to destroy infectivity; this produced no appreciable change in complement-fixation titres of HAA but caused loss of precipitin in crossover electrophoresis tests.

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Reference
Comparison of complement fixation and crossover electrophoresis for detection of hepatitis-associated antigen.
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