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.

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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

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 ≥ 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

- Pringle, R. C., McMichael, S., and Ross, C. A. C. (1971). Immuno-electro-osmophoresis and complement fixation tests for detection of hepatitis associated antigen. *Med. Lab. Technol.*, in press.

Epoxy Resin Sections

Some difficulty in detaching Araldite blocks from glass slides may be experienced by some workers when using the method of Burns (1970). This is avoided by using glass slides as supplied by the manufacturers, ie, neither recleaned nor polished. Alternatively, cleaned slides

may be coated with a thin layer of evaporated carbon (Barnicot and Huxley, 1963).

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Notices

Overseas Tutors' Course in Medical Laboratory Technology

The second of the pioneer series of courses will be held at the Royal Free Hospital and Bromley College of Technology in 1971. The course will offer seven months' training to experienced and qualified laboratory technologists from a number of overseas countries and give them the necessary qualification that they may return to their own countries and set up teaching establishments and examination systems for medical laboratory subjects. Their own technical knowledge will be brought up to date, and they will learn management, teaching technology, and the use of many kinds of visual aids.

This year's tutors, who are from Jordan, Mauritius, Nigeria, Uganda, Malaya, Korea, Vietnam, Thailand, West Indies, Sudan and Malta, are mainly supported by the Department of Overseas Administration.

Further information can be obtained from the Course Director, Pathology Unit, The Royal Free Hospital, Gray's Inn Road, London WC1Y 8UE.

Prize for Biochemical Analysis

The prize of DM 10,000 is given by Boehringer Mannheim and is awarded every two years at the conference 'Biochemische Analytik' in Munich for outstanding work in the field of biochemical

analysis. The prize will be given during the 1972 conference between 25 and 28 April. Papers, either published or accepted for publication between 1 January 1970 and 30 September 1971 may be sent in triplicate before 15 November 1971 to Dr Rosmarie Vogel, Secretary of the 'Preis Biochemische Analytik', D-8000 Munich 15, Nussbaumstr. 20, Germany.

Data on Interference of Drugs with Biochemical Reactions

An information centre which collects data on the interference of drugs with biochemical reactions has been set up. The centre would be pleased to receive information about such reactions which come to light. They would also be prepared to provide advice and information on these matters. All communications should be addressed to Dr B. G. Blijenberg, Dijkzigt Hospital, Rotterdam, The Netherlands.