Letters to the Editor

Temperature Activity of Serum Cholinesterases

In a recent paper (King and Morgan, 1970) the properties of the very rare homozygote for the fluoride-resistant serum cholinesterase could only be postulated. Our subsequent study of two cases of this phenotype $E_1^f E_1^f$ is insufficient to be conclusive but results indicate that predictions regarding the kinetic behaviour of this variant were substantially correct.

With benzoylcholine substrate at 50 μ moles/l maximum activity is obtained at a reaction temperature of 40°C, the Q_{10} (25°-35°C) is 1.30, and the correction factors from 25°C and 30°C to 37°C are respectively 1.33 and 1.14. This represents a divergence of nearly 20% from the atypical homozygote, $E_1^a E_1^a$, for which temperature correction factors were given and, as predicted, would necessitate a separate table of factors for this isoenzyme. While only an indication of these factors can be obtained from two specimens, the matter may take some time to resolve fully, as Lehmann and Liddell (1969) have calculated that the genotype $E_1^f E_1^f$ occurred once in 300,000 of the population and $E_1^f E_1^a$ once in 200,000.

J. KING AND H. G. MORGAN
Department of Biochemistry,
Royal Infirmary, Glasgow, C.A.

References

- King, J., and Morgan, H. G. (1970). The temperature activity relationships of serum cholinesterases. *J. clin. Path.*, **23**, 730-732.
Lehmann, H., and Liddell, J. (1969). Human cholinesterase (pseudocholinesterase): genetic variants and their recognition. *Brit. J. Anaesth.*, **41**, 235-255.

A Rapid Tube Test for Sickling

We were interested in the report by Canning and Huntsman (1970) on the assessment of Sickledex.

We have developed an essentially similar system using known reagents of negligible cost, with minor modifications which make the test more reliable and eliminate the few false positives which occur with the Sickledex preparation. The most frequent need for rapid identification of sickle cell haemoglobin is among possible heterozygotes who although not anaemic, are under risk under conditions of anoxia which may occur, for example, in pneumonia or inadvertently during anaesthesia. The test described is undoubtedly much quicker and more reliable than the microscopic observation of sickling under reduced oxygen tension.

REAGENTS

A stock phosphate buffer is prepared containing 26.0% dry dipotassium hydrogen phosphate (K_2HPO_4) and 10.7% of dry potassium dihydrogen phosphate (KH_2PO_4). The salts should be dried at 120°C for one to two hours before use.

The working reagent is prepared by adding to each 100 ml of stock buffer 5 g sodium dithionite ($Na_2S_2O_4$) and 0.2 g of white saponin (British Drug Houses). The final pH is 6.8. The reagent should be kept in a tightly stoppered container and stored at 4°C. It should be prepared from stock buffer each week. Loss of reducing activity is easily detected during the test by the negative control which fails to produce the purple tint of reduced haemoglobin; the brighter red of oxyhaemoglobin is easily differentiated. Furthermore, after centrifugation with a known positive Hb-S control, instead of a clear straw-coloured solution, some degree of haemolysis is observed (see below).

PROCEDURE

Blood samples taken into sequestrene or heparin or oxalate mixtures are suitable for testing. Approximately 0.02 ml whole blood is added to 2 ml of reagent in a 3 in. $\times$ $\frac{1}{2}$ in. test tube. If the colour pro-

duced by this 1/100 dilution differs markedly from that produced by a sample of normal haemoglobin content, a larger amount of blood should be used to make the colour more nearly approximate. A known Hb-positive blood sample and a normal blood sample should be similarly treated. The tubes are left at room temperature for five minutes and examined for turbidity. The tubes are then centrifuged at about 3,000 rpm for three to five minutes. In severe anaemia, ie, with Hb concentration less than 7g per 100 ml, it is advisable to concentrate the red cell suspension by removing an adequate amount of supernatant plasma before testing.

The result is read by two criteria:
1 Red cells containing Hb-S in the reduced state are resistant to saponin haemolysis and the blood suspension in the reagent remains turbid. A negative result that is, a clear but slightly haemolysed opalescent solution, is seen within five minutes. Reliance on turbidity alone may occasionally give false positive readings where plasma protein concentrations are high as in myeloma (also noted by Canning and Huntsman), or in the presence of severe anaemia where the amount of plasma relative to red cells is high, when turbidity due to precipitation of plasma proteins may occur.

2 Separation of the unhaemolysed Hb-containing cells by centrifugation. After centrifuging the unhaemolysed Hb-S containing red cells rise to the surface leaving a virtually clear straw-coloured solution. The occasional false positives as judged by turbidity alone are eliminated in this way. Ten minutes is required to complete the test. Prolonged standing will produce the same effects as centrifugation.

We have tested numerous samples of various types of heterozygote Hb-S samples and many homozygote Hb-S samples and found no false positive or false negative results by this method when checked against electrophoresis.

M. D. MUSTAFA

Khartoum

J. FIELDING

Haematology Department,
St Mary's Hospital (Harrow Road),
London

Reference

- Canning, D. F., and Huntsman, R. G. (1970). Assessment of Sickledex as an alternative to the sickling test. *J. clin. Path.*, **23**, 736.