Technical methods

Simple method for estimating plasma haemoglobin during open heart surgery

SIDNEY SHAW *From the Department of Haematology, Charing Cross Hospital and Medical School, London*

Standard methods to measure plasma haemoglobin use orthotolidine or benzidine. These have disadvantages of (a) being time consuming, (b) depending for accuracy on critical timing of colour development, and (c) both compounds are considered to be carcinogenic.

Some laboratories give an approximate result, *e.g.*, a one, two, or three plus degree of plasma haemoglobin.

In estimating plasma haemoglobin directly, errors occur associated with varying degrees of turbidity of the plasma. It was found that Lissapol NDB (I.C.I. Ltd.) will clear plasma turbidity with no significant effect on the haemoglobin content.

The following method was found to be rapid, simple, and sufficiently accurate by comparison with standard techniques.

METHOD

Primed pump blood, 9 ml., is taken into 1 ml. of 3.8 g% sodium citrate and 9 ml. of blood after the patient has been heparinized is taken into 1 ml. of 3.8 g% sodium citrate. After centrifugation the supernatant plasma is taken off from each and mixed together in equal amounts.

Mixed plasma, 3 ml., is used for the haemoglobin standard curve and 3 ml. for the blank. To clear the plasma, 7 drops of Lissapol are added to each sample and mixed by inversion several times.

A haemoglobin standard is previously prepared from haemolysed blood with a concentration of 1,000 mg. Hb%.

A calibration curve is prepared by repeatedly adding 0.03 ml. of the above haemoglobin standard and reading after each individual addition in a photoelectric colorimeter using an Ilford green filter no. 625.

Each addition is equivalent to 10mg.% of plasma haemoglobin.

If the optical density of the plasma blank is too high to 'zero' the colorimeter dilute the blank and sample 1 in 2 with ammoniated water and add only 0.015 ml. of the haemoglobin standard for each reading.

To obtain plasma haemoglobin levels during and after the operation use 3 ml. of citrated plasma, add 7 drops of

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Automated screening for sheep erythrocyte agglutination

T. K. MORRIS AND F. J. BYWATER *From the Department of Haematology, Coventry and Warwickshire Hospital*

The routine examination of serum from patients with rheumatoid arthritis using the differential agglutination of sheep erythrocytes is a standard procedure.

With only minor modification the techniques in use are basically those of Rose, Ragan, Pearce, and Lipman (1948) in which sheep cells are sensitized by a potent rabbit anti-sheep serum to a minimum agglutination dose, and used as indicators in a two-fold dilution system of complement-inactivated serum. The differential titre is achieved by a further titration using unsensitized sheep cells. These techniques are time consuming and in routine testing some 60% of sera prove to have a titre of less than 32. The following automated screening technique reduced the number of titrations by detecting sera with titres greater than 16.

The serum under test is inactivated at 56°C. for 30 minutes and samples then placed in autoanalyser standard sample cups. Tests may be run at 40 to 60 tests per hour. Washed sheep cells are sensitized using a rabbit anti-

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Lissapol and mix, read against the blank as above; the result is obtained from the calibration curve (Fig. 1).

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