New micromethod for lupus erythematosus (L.E.) cell tests

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Since the discovery of the L.E. cell phenomenon many methods of carrying out L.E. cell tests have been described. Some micromethods are available, too, but these are either not sensitive enough or do not contain sufficient numbers of blood cells (Snapper and Nathan, 1955; Mudrik, Lee, and Davidsohn, 1961). Micromethods are of importance for experiments on animals and sometimes for routine investigations.

We describe here a micromethod which has a sensitivity similar to normal L.E. cell tests and which may be used with one drop of blood.

METHOD

Put in a cuvette one drop of a heparin solution containing 0.5 units per 0.1 ml. Add to this one drop of blood and mix. Then add two drops of distilled water, to haemolyse the red cells, and traumatize for 60 minutes in a moist chamber as described below. After this traumatization, incubate for 60 to 120 minutes in a moist chamber. Centrifuge in a slide-centrifuge, for 15 minutes. Dry in air and stain with May Grünwald-Giemsa.

TECHNICAL DETAILS

EQUIPMENT FOR CELL TRAUMATIZATION Traumatization is carried out in small Perspex cuvettes, each containing a stainless steel ball activated by a rocking table. The cuvettes are constructed from blocks of clear Perspex, a milled channel in each holding the blood and a $\frac{1}{8}$ in. diameter stainless steel ball. The channel width allows for a total clearance of 0.020 in. between the ball and the channel walls whilst its $\frac{1}{2}$ in. length is predetermined by the speed and degree of deviation of the rocking table. This table, also constructed from Perspex, is operated by an eccentric roller and return spring. The cuvettes are placed on the centre line of the table, their longest side assuming an angle of approximately 80° to its axis. A smear of Vaseline on the table is sufficient to hold the cuvettes in place. The roller is driven by an electric motor at 30 r.p.m. thus giving 60 double deflections of the table per minute. Deflection from the horizontal position is 20° in each direction in the vertical plane. A Perspex moist chamber fits on the table whilst in use.

The cuvette and steel ball are carefully cleaned and

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FIG. 1. Moisture chamber about to be placed on rocking table, with cuvettes containing blood and steel balls in position.

together with the pipette may be sterilized by exposure to ultraviolet light for 20 minutes.

PREPARATION OF MICROSCOPE SLIDES A 'slide-centrifuge' developed from the apparatus of Bots, Went, and Schaberg (1963) was used to collect the traumatized cells onto microscope slides. In this apparatus, which will be described in detail elsewhere, the cell suspension is forced along a narrow tube by centrifugal force. This tube is closed at one end by a microscope slide and a thick filter paper punched with a hole to correspond with the tube outlet. Cells are retained on the slide by centrifugal force whilst the fluid present is drawn away by the filter paper. This method enables easy preparation of microscope slide cell collections from dilute suspensions where only a few drops are available.

COMMENT

We report a new micromethod for L.E. cell tests. We have used this method in man and in mice. It is very important that the blood should be taken under sterile conditions, for example, from the eye veins of mice. By

FIG. 2. L.E. cell phenomenon shown by the method described.

using the slide-centrifuge sufficient leucocytes are collected on the slide. It is important to use heparin, as clotting of the blood inhibits movement of the steel ball and prevents the deposition on the slide of more than a few leucocytes. In an earlier paper we have reported that large amounts of heparin inhibit the L.E. factor, but that 10 units (100 μ g.) heparin to 1.0 ml. blood has no inhibiting effect (Johnson and Bencze, 1964).

SUMMARY

We describe a new micromethod for traumatization of the cells in one drop of blood using equipment developed for the technique. This micromethod has similar sensitivity to that of other methods at present available.

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