Technical methods

Preliminary investigation on a rapid method for the concentration and processing of extracellular blood parasites for electron microscopy

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The microhaematocrit concentration technique for the recovery of microfilariae, trypanosomes, and spirochaetes from the blood has been developed and used by Devignat and Dresse (1955), Wongsathuaythong (1961), Bennett (1962), Woo (1969 and 1970), Goldsmid (1970), Goldsmid, Mahomed, Makanji and Muir (1972), and Goldsmid and Mahomed (1972). The last authors also suggested its use for the concentration of *Borrelia duttoni* for electron microscopy.

Most methods of concentration and fixation of trypanosomes and *Borrelia duttoni* for electron microscope studies are comparatively long and involved procedures (Vickerman, 1962 and 1969; Demaree, 1970) but the method described is extremely rapid and easy and preliminary studies have given very encouraging results.

Methods

Blood obtained by cardiac puncture from rats and mice infected with *Trypanosoma (Trypanozoon) brucei* or *Borrelia duttoni* was collected into sodium citrate. Samples of this blood were then drawn into microhaematocrit capillary tubes and then one end was sealed in a flame. The tubes were then centrifuged at 12 000 rpm for two minutes in a Hawksley microhaematocrit centrifuge as described by Goldsmid (1970).

After concentration, the haematocrit capillary tube was immediately removed and scored with a diamond knife approximately 0.5 mm above the top level of the buffy coat and the tube broken using forceps. A second score was made 1 mm below the

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Fig. 1 Diagram to illustrate positions of the score marks with diamond knife.

P Plasma, B Buffy coat, E Packed erythrocytes

bottom level of the buffy coat and the tube broken again (Fig. 1).

The short length of tube containing the buffy coat and part of the layer of packed erythrocytes was then immersed in 4% glutaraldehyde in phosphate-buffered saline pH 7.2. The tube containing the fixative was then shaken vigorously to ensure that any small air bubbles trapped in the haematocrit capillary tube were displaced. After 20 minutes the packed red cell layer had become firm enough to allow the plug of material in the capillary tube to be gently forced out with a piece of wire into fresh 4% glutaraldehyde. The specimens were fixed for 60 minutes at room temperature in the second change of glutaraldehyde.

After washing in phosphate buffer, the plug was post-fixed in osmium tetroxide buffered to pH 7.3 at 3°C for 60 minutes to the formula of Rhodin (1954) and Zetterqvist (1956) as described by Kay (1961). The plug was then dehydrated in ethanol and embedded in Epon (Luft, 1961, as quoted by Mercer and Birbeck, 1961) after which the blocks were sectioned using a glass knife.

Orientation of the plug was simple since the buffy coat was easily distinguished from the erythrocyte layer.

Results

The results are shown in Figures 2, 3, 4, and 5. The concentration of *B. duttoni* in relation to the buffy coat of leucocytes is shown in longitudinal section in Fig. 2 while the degree of concentration of *T. brucei* is illustrated in Fig. 3. Figures 4 and 5 show

Fig. 2.

Fig. 3.

Fig. 2 *Electron micrographs illustrating the concentration of B. duttoni in relation to the buffy coat of leucocytes* $\times 9300$

Fig. 3 *Electron micrograph illustrating the degree of concentration of T. brucei* $\times 9300$

Fig. 4 *Electron micrograph. Section of T. brucei* $\times 27\,000$

Fig. 4.

Fig. 5 Section of flagellum of *T. brucei* $\times 52\,000$

high-power electron micrographs of *T. brucei* to illustrate detail on trypanosomes treated by this method.

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