

## Technical methods

### Separation of haemoglobins Lepore and H from A and A<sub>2</sub> in starch or acrylamide gels buffered with tris-E.D.T.A.

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Owen and Got (1957) first used the high resolving power of the starch gels of Smithies (1955) to separate foetal and adult haemoglobins. Unfortunately some haemoglobin separations, notably that of Lepore from A and A<sub>2</sub>, are difficult to achieve in gels made from buffers containing high concentrations of borate, possibly because of some degree of binding between the haemoglobins and borate ions. Recently Lehmann and Sharih (1961) described separation of Lepore haemoglobin on paper in tris-E.D.T.A. buffer. We have found that this buffer, used at a lower ionic strength, gives an excellent resolution of Lepore in starch gels, which have the advantage of a much narrower zone on which to apply the samples than paper, and can, after clarification with anhydrous glycerol (Gratzer and Beaven, 1960), be scanned photometrically without staining. Alternatively water-clear gels can be made from a polymer of 5% N-N bis-acrylamide and 95% acrylamide similar to those described by Raymond and Weintraub (1959).

The separation of haemoglobins Lepore, A, and A<sub>2</sub>

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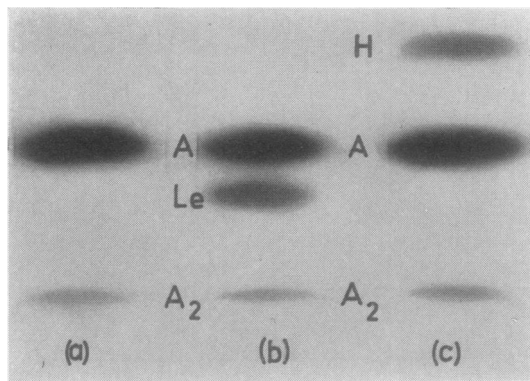


FIG. 1. Electrophoresis of (a) normal, (b) Lepore, and (c) H containing haemolysates in acrylamide gels in tris-E.D.T.A. buffer.

To facilitate photographic reproduction the gels have been stained with naphthalene black and photographed with a red filter. Normally the gels are scanned unstained.

is shown in Fig. 1 (b) and of H, A, and A<sub>2</sub> in Fig. 1 (c). The patterns on photometric scanning at 540 m $\mu$  are shown in Fig. 2A and B.

The starch gels were made from Starch-hydrolysed (lot 154) purchased from Connaught Laboratories, Toronto, Canada, and the tris-E.D.T.A. buffer described by Cradock-Watson, Fenton, and Lehmann (1959), diluted 1 in 3.

The acrylamide gels were made from chemical grout AM9 supplied by Cynamid (Australia). The gels were prepared by polymerizing 7.5 g. of monomer per 100 ml. of solution according to the manufacturer's instructions.

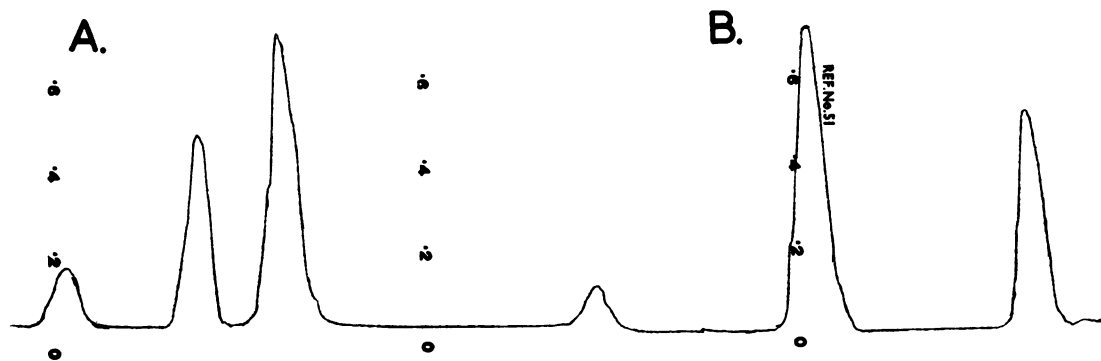


FIG. 2. A, Photometric scan at 540 m $\mu$  of the pattern shown in Fig. 1a, and B of that shown in Fig. 1b.

The gels were equilibrated with three changes of excess tris-E.D.T.A. buffer for 24 hours before using.

The samples were inserted in both types of gel on slips of Whatman 3 mm. filter paper. The runs were carried out at a potential gradient of 8 volts per cm. at room temperature (approximately 16°C.) for six hours in an apparatus based on that of Smithies (1955). The top and bottom 1.5 mm. of the starch gels were sliced off and the centre section clarified in anhydrous glycerol and scanned between two microscope slides in a recording microdensitometer (Curtain, 1960). A 540 m $\mu$  second order Bausch and Lomb interference filter was placed in the light source of the low-powered ( $\times 80$ ) optical system used. The clear acrylamide gels were scanned uncut.

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## A portable water-bath for use when investigating the coagulation mechanism

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The recent production by Luckham Ltd. of a portable water-bath has overcome the previous difficulty of controlling anticoagulant therapy at the bedside. The bath has been used at St. Helier Hospital for some time

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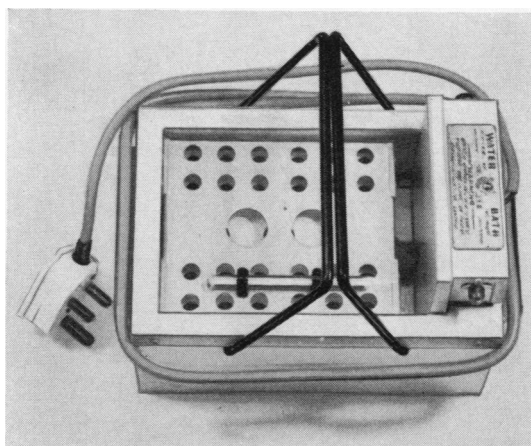


FIG. 1. Apparatus without lid and handles in carrying position.

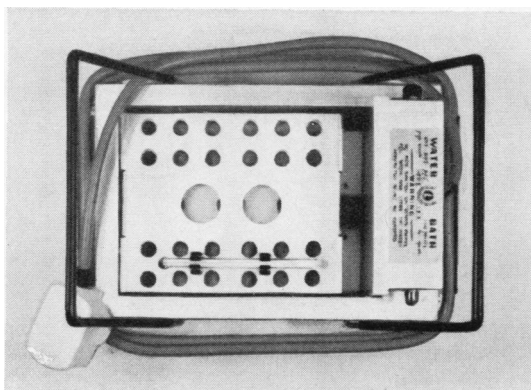


FIG. 2. Apparatus without lid and handles retracted ready for use.