

capillary orifice furthest away from the solution is sealed in a micro burner. After cooling, the solution is centrifuged towards this sealed end. The two capillaries thus filled are then placed in a suitable glass tubing about 10 cm in length and 5 mm in diameter (C). They are put in place by means of a wad of glass wool or cotton (D). The tube is then evacuated to about 15 mm pressure and is then sealed by means of an ordinary Bunsen burner. The tube may once more be subjected to centrifuging in order to have a clean-cut meniscus of each of the solutions contained in the capillaries. By means of adhesive tape (G) the tube is then attached to a rectangular glass plate, which is about 12 cm long and about 4 cm wide (E) and which possesses a hairline or a scratch across its width (F). The plate thus mounted is then placed into a water bath which is kept at room temperature to within  $\pm 3^\circ$  C. After four days, the distance between the two menisci of the two solutions and the scratch, or hairline on the plate, is measured under a low-powered microscope possessing a micrometer scale in the eyepiece. These measurements are repeated subsequently once a day for a week. Thus it is easily ascertained which of the two solutions lost less solvent, this being the solution of higher molarity.

The molarities of the standard (azobenzene) employed are 0.05, 0.1 and 0.15 and the most suitable concentrations of the unknown sample are between 1 and 3 per cent. Differentiation between  $\pm 0.01$  molarities is possible.

The method described precludes any possibility of mixing of the two solutions and also permits the recovery and re-use of the unknown, while the results,<sup>3</sup> which will be published in detail in an appropriate analytical journal, compare favorably with the original Barger method or any of the known modifications thereof. The weighing out of the sample may be done on a macro, semi-micro or micro scale.

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### A SIMPLE STAIN FOR TISSUE CULTURES

ANY one confronted with the necessity of staining hanging-drop tissue cultures grown in plasma has been impressed with the difficulty of securing a cytoplasmic stain which would not also tint the plasma of the clot to such intensity as to obscure largely the delicate cytoplasmic contours of the cells.

Many stains have been tried in this laboratory for coloring tissue cultures *in situ* in the plasma clot. The following simple method has given consistently good results on countless cultures over a period of nearly a year. It was developed to stain particularly cultures

of brain tissue in which it reveals with a surprising clarity the finest processes of the cells as well as the nuclei and certain cytoplasmic inclusions.

### METHOD

- (1) Remove all paraffin and vaseline from the coverslip with cotton pledgets soaked in chloroform.
- (2) Fix in 10 per cent. neutral formalin or absolute alcohol for 24 hours.
- (3) Place in 1 per cent. aqueous solution of Toluidine blue for 1 hour.
- (4) Wash in two changes of distilled water.
- (5) Dehydrate in 85 per cent. alcohol 2-3 minutes.
- (6) Place in 95 per cent. alcohol 2-3 minutes.
- (7) Transfer to absolute alcohol until the clot contains little stain. This step may be controlled by watching the decoloration under a microscope. The differentiation takes 5-10 minutes.
- (8) Clear in xylol.
- (9) Mount in balsam or Nevillite.

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<sup>3</sup> A. M. Levy, M.Sc. Thesis, New York University, Graduate School, April, 1940.