FIG. 1.—Undecalcified section mounted in water: (a) unstained, (b) stained with silver nitrate. The black calcified bone in (b) closely corresponds with the calcified area in (a). The soft tissue structure is preserved; in both (a) and (b) the osteoid seam surrounding the calcified trabecula is obvious. $\times 120$.

disappearance of the red calcium lake formed in sections covered with aqueous alizarin (pH 2.5). Alizarin buffered to neutrality has not proved a useful calcium stain. Most information is obtained by staining some sections by the silver-safranin method and others by haemalum and eosin with or without previous silver staining.

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A Rapid Method for Estimating Fibrinogen in Plasma

M. COLES AND W. ROMAN

From the Biochemistry Division, Institute of Medical and Veterinary Science, Adelaide, South Australia

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The letter by Dr. G. L. Robinson in the *British Medical Journal* (1956) encourages us to publish our experience with the turbidimetric method of Parfentjev, Johnson, and Clifton (1953) which Fowell (1955) elaborated, but which does not seem to be known in either the U.K. or Australia.

With the availability of fibrinogen solutions for intravenous administration, the rapid estimation of fibrinogen in plasma, especially in post-partum haemorrhage or conditions associated with fibrinogenopenia, becomes of ever-increasing importance as has also been shown by FitzGerald and Jackson (1956). The classical method, still used by FitzGerald and Jackson, is too cumbersome and takes too long in emergencies and none of the so-called bedside tests are wholly satisfactory, as Dr. Robinson points out in his letter. The turbidimetric method can be carried out by any technician who can use a colorimeter in 10 to 15 minutes and requires only a simple colorimeter which is available these days in all hospital laboratories. We use either the "unicam" SP 300 with the blue-green filter or SP 400 at a wavelength of 500 $m\mu$.

Parfentjev *et al.* found that only fibrinogen is precipitated from plasma with 20% saturated ammonium sulphate solution and that 1 part of plasma or diluted plasma with 9 parts of an ammonium sulphate solution containing 133 g. litre will give a final ammonium sulphate concentration of 12% which causes a turbidity proportionate to the fibrinogen concentration. We found that this turbidity obeys Beer's law over the range 0.05–1.0% and direct proportionality between optical density and fibrinogen concentration is obtained after deduction of a blank value due to the natural turbidity of the plasma under test when using the above-mentioned wavelengths and colorimeters.

Method

Citrated or oxalated blood is used and centrifuged at 2,000 r.p.m. for five minutes, the plasma taken off and recentrifuged under the same conditions. Two test tubes are set up, the blank containing 0.5 ml. of plasma and 9.5 ml. normal saline, the test 0.5 ml. plasma, 0.5 ml. normal saline, and 9 ml. ammonium sulphate reagent. Both tubes are mixed by gentle inversion (no shaking, since this may lead to partial defibrination and uneven turbidity). Exactly three minutes (timed with a stopwatch) after the addition of the ammonium sulphate to the plasma under test, its optical density is read in the

colorimeter which has been set at 0 optical density with water. Then the blank is read and the blank value deducted from the test value, the difference giving the turbidity due to fibrinogen. The fibrinogen concentration of the plasma can be read from the standard graph giving fibrinogen concentration against optical density.

The only reagents used are normal saline and Parfentjev's ammonium sulphate solution prepared by dissolving 133 g. $(\text{NH}_4)_2\text{SO}_4$ + 10 g. NaCl in 1 litre distilled water preserved by the addition of 0.025 g. merthiolate. The standard graph is best prepared by using pooled plasma whose fibrinogen content has been determined by the classical method. Only two points are required when using the above-mentioned instruments and wavelengths, since with these a straight line will be obtained. The use of pure fibrinogen is not recommended, as the complete reconstitution of freeze-dried fibrinogen without appreciable turbidity or loss due to defibrination is not possible in our experience.

The normal fibrinogen content of plasma has been found to vary between 0.113 and 0.385 g./100 ml. with an average of 0.246 g./100 ml., but may be as high as 0.5 g./100 ml. in pregnant women. When the fibrinogen content in post-partum haemorrhage falls below 0.05 g./100 ml., intravenous administration of fibrinogen was found to be indicated, while patients with values of about 0.1 g./100 ml. have responded to transfusion of whole blood alone.

Summary

Technical details for the rapid estimation of fibrinogen applying the turbidimetric method of Parfentjev *et al.* to English colorimeters are given. This method allows the estimation of 0.05 to 0.5 g. fibrinogen/100 ml. plasma with an accuracy of ± 0.013 g. fibrinogen/100 ml. in 10 to 15 minutes. The method can be carried out by any trained technician in a small hospital laboratory and is accurate and quick enough for emergencies.

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A Simplified Method for Polarized Light Observations with the Binocular Microscope

D. M. McCLURE

From the Area Laboratory, the General Hospital, South Shields

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During the past decade the use of polarized light has become an established accessory to clinical microscopy, and with its general adoption there has been a progressive simplification of the equipment employed. The specially equipped polarizing microscope and the Nicol prism have been wholly replaced by the use of "polaroid" plastic, and with this the factory-made polarizer and analyser have in turn given way to the improvised article.

The recommended procedure for providing these has been largely standardized. A disc of "polaroid" sheet is cut to fit the substage filter carrier, and a second smaller disc is incorporated in a cap to fit over the eyepiece. With a monocular microscope this is wholly satisfactory, but it is quite inapplicable to the binocular instrument. In passing through the system of prisms, the polarization of the light is almost totally lost, and an eyepiece analyser produces only a negligible light extinction. In practice, therefore, one is obliged to change over to a monocular body, and, while with most instruments this is a simple enough procedure, it does constitute a minor inconvenience. There are, moreover, some which lack provision for this change-over.

FIG. 1.—Sectional diagram of screw-cap analyser in working position.

This disadvantage can be completely overcome by the very simple expedient of placing the analysing polaroid screen not over the eyepiece but directly over the slide under examination. Certain objections to this come to mind at once—interference with the definition of the image and scratching of the plastic material in the course of usage. Of these, the first can be discounted (with one exception to be specified) and adequate precautions can be taken against the second. The principal inherent limitation in this method is that it is not suited