TABLE II

RESULTS IN GUNN RATS

Plasma Total Bilirubin Level (mg/100 ml)	Optical Density	
	Before	After Boiling
6.0	0.079	0.301
11.6	0.218	0.821
7.0	0.205	0.484
8.8	0.157	0.562

COMMENT

The addition of heating to the Sanford technique causes opalescence only when the test plasma contains a raised level of unconjugated bilirubin. The opalescence may be difficult to detect and in such sera an initial optical density should be recorded after heating and before the addition of bathophenanthroline. The nature of the opalescence is uncertain. Bilirubin added to standard iron solutions did not produce opalescence whereas it occurred with similar concentrations of bilirubin in plasma, suggesting that a bilirubin complex is involved. The effect was not observed with plasma containing conjugated bilirubin, which is surprising, since conjugated bilirubin is known to be split to free bilirubin and glucuronic acid on heating. The liberated glucuronic acid may inhibit the opalescence which would explain why it was not found with plasma from patients with obstructive jaundice, where a significant amount of unconjugated bilirubin is also present. Alternatively, the liberated bilirubin may differ from that present in indirect reacting sera and from crystalline bilirubin in being one of the modified forms postulated by Fog and Bakken (1967).

We would like to thank Dr D. C. Nicholson for much helpful advice.

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Improved automated methods of protein determination

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This paper investigates the technique of Lowry, Rosebrough, Farr, and Randall (1951) with a view to improving on current automated techniques.

An automated method of protein determination using Folin and Ciocalteu phenol reagent has been described by Technicon Instruments Ltd (1961) which resolves protein concentration down to 50 µg protein/ml at the very best and does not give a linear function with concentration. The techniques described in this paper resolve protein concentration down to 0.2 µg/ml and obey Beer's law over a reasonable range. A method giving high sensitivity offers distinct advantages when monitoring fractions after column separation or gel filtration, especially when dealing with small samples of lymph, plasma, etc., such as are encountered in paediatric practice. Hitherto these samples have necessitated laborious and often inaccurate manual dilution.

REAGENTS

The Folin and Ciocalteu reagent was supplied by Fisons Scientific Apparatus Ltd. It was standardized to the appropriate normality after determining the acid concentration by titrating against $\frac{N}{1}$ sodium hydroxide to *pH* 7.0 on a *pH* meter. Suitable additions of water were then made. It was finally decided to use 0.5 N Folin and Ciocalteu reagent (see results). Two per cent sodium carbonate in 0.1 N sodium hydroxide and 0.5% copper sulphate in 1% sodium tartrate, brought to *pH* 7.0 with sodium hydroxide, were used. Solutions were all prepared from analytical grade reagents.

STANDARDS

Primary standards were prepared from crystalline bovine albumin to give ranges of 0.1 to 500 µg/ml protein and from 0.5 to 8 g/100 ml protein in isotonic saline.

Working standards were prepared by diluting Versatol standards (William Warner & Co. Ltd, Eastleigh, Hants).

METHODS

Manifolds were set up for a TechniconAuto Analyzer (Technicon Instruments Ltd, Hanworth Lane, Chertsey, Surrey), as shown on the flow diagrams (Figs. 1 and 2).

The rate of sampling in each case was 50 per hour

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FIG. 1. AutoAnalyzer manifold for high sensitivity protein measurement.

FIG. 2. AutoAnalyzer manifold for high concentration protein measurement.

FIG. 3. The effect of wavelength in the Lowry reaction.

FIG. 5. Limits of resolution with high sensitivity manifold.

using a 1 : 1 wash/sample ratio and a cam for use on sampler II was made for this purpose.

Particular care was necessary in constructing the high concentration manifold as pressure may build up at the post-pump cactus G.O. Also care must be exercised in sampling with the microbore sample tube to minimize the chance of blockage. However, as the volume of aspirate from each sample is only 18 μ l there is no necessity for the probe to enter the sample to any significant depth.

RESULTS

The optimum wavelength for measuring the coloured complex was found to be 750 m μ (Fig. 3).

Experiments performed to ascertain the optimum concentration of Folin and Ciocalteu phenol reagent showed quite clearly that 0.5 N Folin and Ciocalteu gave the best results in all cases (Fig. 4). This was particularly so with increased temperature.

The maximum limit of resolution obtainable using the high sensitivity manifold is shown in Fig. 5, and

FIG. 4. The effects of temperature and Folin and Ciocalteu reagent on sensitivity.

A = 20°C B = 30°C
C = 40°C D = 50°C

FIG. 6. Calibration curve for high protein concentration.

Fig. 6 shows the calibration curve for the high concentration protein method.

Samples analysed after separation by gel filtration gave results which when totalled gave 94 to 102% of the original.

COMMENT

Both methods gave excellent reproducibility and, particularly with the high sensitivity method, it was found that sample range could be varied by changing the sample volume aspirated and if necessary running it at 20°C, thus obviating full-scale deflection readings.

The improvement in sensitivity with this method appears to result from the reaction with the phenol reagent, the initial biuret reaction appearing to go to completion very rapidly, neither time nor temperature within the conditions described having much effect.

It is interesting to note that the reaction with 0.25 N phenol reagent appears to be independent of temperature, a phenomenon which is still being investigated.

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Micro modification of the dilute blood clot lysis time for determining fibrinolytic activity

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Methods in general use for measuring circulating fibrinolytic activity include the dilute blood clot lysis time (Fearnley, Balmforth, and Fearnley, 1957) and the euglobulin lysis time (von Kaulla, 1963). This paper gives details of a micro modification of the first of these which can be carried out on capillary blood and has been found useful in demonstrating increased fibrinolytic activity in children, small animals, and in surveys where serial determinations are required.

APPARATUS AND REAGENTS

WHITE CELL DILUTING PIPETTES Standard white cell pipettes are treated with water-soluble silicone solution (Siliclad, Clay Adams Inc. NY). These pipettes are chilled by placing them in a 6 × 1½ in. boiling tube immersed in melting ice.

PHOSPHATE BUFFER, pH 7.4 KH₂PO₄, 3.02 g, dissolved in 250 ml distilled water, is added to 9.47 g Na₂HPO₄ dissolved in 1 litre of distilled water. This buffer is stored at 4°C.

THROMBIN-BUFFER REAGENT Bovine thrombin (Leo Pharmaceutical Co.) is added to phosphate buffer to give a final concentration of 5 NIH units of thrombin per millilitre of buffer. This reagent is made up freshly on the day of use and stored at 4°C.

METHOD

A free flow of capillary blood is obtained by pricking a finger tip or ear lobe or heel. Blood is drawn up to the '1' mark of a chilled white cell diluting pipette and diluted with cold thrombin-buffer reagent to give a final dilution of 1 in 11. After rapid mixing the contents of the pipette are blown out into a 3 × ¾ in. glass test tube. This tube is placed in melting ice for 20 minutes to allow a clot to form. It is then transferred to a waterbath at 37°C and examined at intervals for lysis. The length of time between placing the clot at 37°C and complete disappearance of the clot is the lysis time.

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