necessary, 2 drops of Tween 20 per litre may be added to the water in the recipient stream to ensure a good bubble pattern. Thirty determinations per hour are performed by operating the sampling module at 60 per hour and alternating samples with cups of water.

Colour development varies with the ambient temperature and when large numbers of estimations are performed standards should be run about every two hours. This may not be necessary if a constant temperature bath regulated at 25°C. is used. Colour development is poor at 37°C.

The results are read from the graph constructed from the standards using a chart reader and making allowance for dilution.

DETERMINATION OF GLUCOSE IN BLOOD AND CEREBROSPINAL FLUID

CAPILLARY BLOOD OR C.S.F. Add 0.2 ml. blood or C.S.F. to 0.8 ml. 0.1% formaldehyde solution in a sample cup. This permits of two determinations being made on each sample if required, or allows for further dilution when values above 500 mg./100 ml. are encountered; when this is unlikely 0.1 ml. blood diluted with 0.4 ml. formaldehyde suffices.

VENOUS BLOOD OR C.S.F. Heparinized or oxalated blood or C.S.F. may be preserved by the addition of 0.02 ml. 20% formaldehyde per ml. (the error due to dilution is insignificant). The blood or C.S.F. is diluted 1 in 5 with water when convenient.

Sodium fluoride in amounts sufficient to prevent loss of glucose through glycolysis was found to inhibit the colour production to a variable extent. Formaldehyde in the quantities used prevents loss of glucose for at least 24 hours even at 37°C., and does not interfere with the reaction.

The reproducibility of the technique was determined by performing 10 estimations on a single blood sample. The mean glucose level was 99.1 mg./100 ml. Standard deviation was 1.73 mg./100 ml. (range 97 to 102 mg./100 ml.). In a series of experiments the recovery of added glucose was 97 to 104%.

Using the chart reader, glucose values between 12.5 and 500 mg./100 ml. can be read accurately; over most of this range Beer's law is complied with.

DETERMINATION OF GLUCOSE IN URINE

The presence of inhibitory substances in urine which are not removed by dialysis makes it necessary to decolorize with activated charcoal before analysis. When amounts of glucose less than 100 mg./100 ml. are present, add 20 to 50 mg. activated charcoal to about 2 ml. urine in a sample cup, stir, and allow to settle. If larger quantities of glucose are present the urine is first decolorized and then diluted with water to give a final glucose concentration of 5 to 100 mg./100 ml.

The recovery of added glucose from urine was 93 to 112%.

A micro technique for protein concentration suitable for quantitative electrophoresis of cerebrospinal fluid

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A simple apparatus has been devised for concentrating 0.5 ml. samples of cerebrospinal fluid sufficiently to enable quantitative electrophoresis for proteins on cellulose acetate to be undertaken subsequently (Colover, 1961).

It uses a principle described by Miyamoto, Anan, Arai, Sakagiski, and Nomiya (1957) and Kohn (1959). The method is designed to avoid precipitation of proteins or drying them on a membrane, so that they may be recoverable in maximal amounts with the minimum of qualitative or quantitative change. It might thus be suitable for immunoelectrophoresis or for concentrating other biological fluids available in small quantities. Tests on one of the early prototypes of the apparatus showed that 81% to 86% of the protein, estimated by the Lowry, Rosebrough, Farr, and Randall (1951) method was recovered from it. The more recently developed chamber described here is an improvement on this earlier type as

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FIG. 1. The concentration chamber (not strictly to scale.)

FIG. 2. Method of adjusting projection of tube by means of a key of known thickness. After this has been done, the key is removed and the top plate, B, is placed on to the base plate, A (Fig. 1).

can be gauged by the increased density of the bands after electrophoresis.¹

The chamber used (Fig. 1) is made of transparent resistant plastic (Perspex) and glass and is composed of three parts. It has a square base plate (A) whose dimensions are 4 cm. $\times$ 4 cm. $\times$ 1.25 cm. This plate contains a cylindrical well 2.2 cm. in diameter, of which the side wall is 6 mm. deep. The bottom of this well is shaped as part of the surface of a sphere, and in it is cut a circular groove (D) 0.5 mm. deep. The internal diameter of the groove is 1.0 cm. and its external diameter is 1.2 cm. On the base plate rests a square top plate (B), of which the dimensions are 4.0 cm. $\times$ 4.0 cm. $\times$ 0.3 cm. similarly made of plastic material. The centre of the top plate is pierced by a circular hole 1.25 cm. in diameter. The third part of this apparatus is a glass tube (C) about 7 cm. long, having an internal diameter of 1.1 cm. and a constriction about 1.8 cm. from the lower end. This end of the tube is closed by means of a cellulose (Visking) membrane made from tubing 1 5/8 in. diameter which has been opened by cutting one side, and moistened in conductivity water. The membrane is secured to the glass tube by means of a retaining rubber band on the constriction, and should be taut. By means of a key (Fig. 2) the tube is made to project 6.25 mm. through the top plate (B).

Into the lower chamber (Fig. 1) is pipetted 0.5 ml. of cerebrospinal fluid. In the upper chamber is placed 1 ml. of 30% w/w polyethylene glycol solution in distilled water (Carbowax 20 M., G. Gurr). The two plates are

¹The commercial production of this apparatus is being undertaken by Messrs. Electrothermal Engineering Ltd. The design has been patented by the N.R.D.C.

kept close together by means of rubber bands and the apparatus is put in a moist chamber to prevent evaporation and kept at 4°C. for 16 hours overnight. After this period the cerebrospinal fluid has been concentrated to 5 to 20 μ l., most of which lies in the circular groove. In earlier trial chambers which did not have such a groove it was found that a ring of viscid protein collected often in the base of the well opposite the edge of the glass tube. (Staining the membrane from the chamber with Ponceau S after the concentration process was completed showed little or no protein adhering to it.) To the concentrate is added 5 μ l. of barbitone buffer pH 8.6, ionic strength 0.05, and this is then transferred to a cellulose acetate membrane for electrophoresis by the method of Kohn (1957). After staining the bands with naphthalene black, the strip is scanned in an EEL scanner connected externally to a Unigalvo spot galvanometer. In this way it has been found possible to examine fluids containing as little as 15 mg. % of protein. Owing to the small amount of fluid required, the reproducibility of results obtained in replicate experiments can be investigated.

The method and results of a survey of the protein pattern of cerebrospinal fluid in multiple sclerosis and other neurological conditions will be published in detail in due course (Colover, 1961b).

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