CCXIII. ULTRAFILTRATION AND CONCENTRATION BY ULTRAFILTRATION BY A CENTRIFUGE METHOD.

By ROBERT BRINKMAN AND JOHAN VAN STEINFOORN.

From the Biochemical Laboratory, Groningen, Holland.

(Received 4 June 1936.)

“ULTRAFILTRATION is an exceedingly slow and tedious operation and at present restricted to the laboratory.” This statement, in a recent text-book of colloid chemistry [Thomas, 1934], will be more or less endorsed by most experimental workers, especially by those who only use this method occasionally. Concentration by ultrafiltration might seem a choice method for colloids which are inactivated easily, but so far it has only been successful in few cases. The ineffectiveness of this technique is principally due to clogging of the ultrafilter membrane by a deposit of the disperse phase; this reduces the filtration velocity very much or stops the process altogether. In many methods a scraping device is incorporated, but its advantage is doubtful, since a very thin deposit is as effective in retarding ultrafiltration as a thick one, and the scraper or stirrer cannot effectually remove it. Hence as a rule a small amount of ultrafiltrate is obtained in the first hours and after that not much more. Of course, an experienced worker can make membranes, which combine impermeability to protein with rapid ultrafiltration in the first hours, but for clinical application, especially, there is need of a method for collecting about 3 ml. of protein-free ultrafiltrate from about 10 ml. of serum or blood in half an hour's time, without requiring skill in the preparation of suitable membranes. Such a method is described in this paper.

The pressure necessary for rapid ultrafiltration is obtained by centrifugal force, as in De Waard's [1918] method. In this latter method however the filtering membrane is, as in all other procedures, at right angles to the direction of the filtering solutions, so that the membrane is clogged by the deposit in about 1 hour.

If however one uses a small cylindrical unglazed porcelain pot, covered on the inside by an ultrafilter membrane, the centrifugal pressure is exerted on the sidewall and bottom, whilst any peripheral part of the liquid which becomes increased in specific gravity is centrifuged towards the bottom. In this way clogging is prevented, since any part which becomes denser through ultrafiltration moves at right angles to the direction of the ultrafiltering stream.

The velocity of ultrafiltration now only depends on the centrifugal pressure, and the dimensions of the column of solution. The following example illustrates the situation: an ultrafilter tube is filled with 10 ml. of 1 % haemoglobin solution and centrifuged for 10 min.; in this time 2.1 ml. of protein-free ultrafiltrate are formed; a second rotation for 10 min. yields 1.3 ml., a third yields 1.0 ml. and a fourth 0.8 ml. So in 40 min. 5.2 ml. of ultrafiltrate have been obtained from 10 ml. of haemoglobin solution, the concentration of which is now doubled.

If the amount of Hb solution in the cylinder is brought up to 10 ml. at the end of each 10 min. interval, the production of ultrafiltrate proceeds at a constant rate of 2 ml. per 10 min.

As regards coating the porcelain cylinder with an ultrafiltration layer, it was desirable that (a) the membrane should be impermeable to colloids like serum proteins or haemoglobin, without at the same time being too slowly permeable to water and crystalloid solutes, (b) the ultrafilter layer should be easy to make and reproducible, and (c) the durability of this layer should be as good as possible or that the layer might be quickly regenerated. After many trials we found these conditions to a certain extent fulfilled by covering unglazed "chamotte" cylinders (which have fine pores and a smooth inner surface) with a layer of sodium water-glass (30%). Potassium water-glass cannot be used. The membrane is made as follows.

The dry porous cylinders are filled with the water-glass and left standing for some minutes; the water-glass is then poured out again and the cylinders are kept upside down till no more liquid drains off. They are then set aside for 30 min. at room temperature. After that, they are rinsed with tap water for 1 to 2 min. and tested with haemoglobin solution.

For a 10 ml. covered cylinder filled with 1% haemoglobin solution and rotating in an ordinary centrifuge at 3000 r.p.m. the amount of ultrafiltrate in 10 min. should be 1–2 ml. If, at the first trial the amount of protein-free ultrafiltrate is too small, e.g. 0.5 ml., this is corrected by rinsing with tap water for 10–15 min. The silicate covering appears to swell a little under this treatment and the permeability increases. If the contact with streaming water is too long, the swelling may lead to a permeability for colloids; the filters have then to be dried for a longer time, e.g. overnight, and generally the impermeability for colloids is restored. If this is not so, one has to apply a new coating of water-glass.

We have found that some porous cylinders are not suited for these water-glass membranes. We use a type having a soft and smooth surface with very fine pores; it is not known by any special name, but may be obtained at low cost from Messrs Marius, Utrecht, Holland. The filter tubes will stand a long and intensive use; we have used them for 4–5 hours every day, for 2 weeks at a time, without a significant change in permeability. Sometimes a slight decrease was observed, which was overcome by a short rinsing with tap water.

When not in use the filters are kept in moist paper in a cool place. They may then dry a little too much and consequently have a lessened permeability; this is treated as described above. It is remarkable that no deposit at all becomes observable when the filters are used for concentration by ultrafiltration. The concentration of the disperse phase steadily increases, and so it is possible to divide 10 ml. of a 1% haemoglobin solution into 5 ml. of a 2% solution and 5 ml. of a protein-free ultrafiltrate in about half an hour.

If, during this process, the volume of the contents of the tube is kept constant every 10 min., by replacing the lost water by small rods of a suitable solid, inert material, a 10-fold concentration may be reached in 1 hour. We use for this purpose an artificial resin, but any other solid with a specific gravity just over 1 may be used.

When larger amounts of colloidal solution are to be concentrated, one can use 4 tubes at a time, keeping them filled every 10 min. and finally reducing the number of tubes to 3, 2 and 1 when the total volume is reduced to 10 ml. We have e.g. concentrated 200 ml. of urine, containing 0.04% of Bence Jones protein to 4 ml. of a 2% solution in 5 hours' time. It must be mentioned that the porous wall of the cylinder we used can take up about 2 ml. of solution, so that, if the ultrafiltrate is required for analyses, the first 2 ml. must be discarded. Owing to the rapid ultrafiltration process this takes 10–15 min., so that not much time is lost.

SUMMARY.

By coating porcelain cylinders with water-glass, ultrafilters are obtained which are very suitable for ultrafiltration and concentration by ultrafiltration by means of the centrifuge. The filters are not clogged by the disperse phase and will stand heavy use.

REFERENCES.

- Thomas (1934). Colloid Chemistry (New York and London: McGraw-Hill Book Company).
De Waard (1918). *Arch. néerl. Physiol.* 2, 530.