CXLIV. A RAPID METHOD FOR OBTAINING PROTEIN-FREE ULTRAFILTRATES OF BLOOD AND PLASMA.

BY CLIFFORD WILSON AND ENSOR ROSLYN HOLIDAY¹.

From the Medical Unit Laboratories, The London Hospital.

(Received June 20th, 1933.)

SINCE Smith [1928, 1, 2], working in these laboratories, developed a rapid method of ultrafiltration of plasma, we have had occasion to use the method frequently and on a large scale.

The following is an account of modifications in the technique which we have found necessary, (1) to improve the rate and yield of filtration, (2) to give membranes reliable and simple in preparation.

The chief difficulties experienced in using fine grade collodion membranes are firstly that they are very slow, and secondly that after a short time the rate of filtration is markedly decreased owing to clogging of the filter pores.

We have aimed at obtaining a membrane which will hold back protein and will filter rapidly at low pressure differences. There are two disadvantages of high pressure filtration. (a) The filters clog much more quickly; (b) the structure of the membrane may be actually altered so that its pore size is changed. With these considerations in mind we work at a pressure difference of about 70 cm. mercury. We prefer using a negative pressure, as it is more convenient in use and more economical, any good water suction-pump giving adequate pressure difference.

Methods devised to relieve blocking of the membranes are many. Pierce [1927] used shaking, Smith [1928, 1, 2] used a rocking filter with beads on the membrane, Elford [1931] used alternation of pressure. For filtration of fluids such as plasma we believe that the method described here gives very much more satisfactory results than any of the above.

METHOD.

The apparatus (see Fig. 1) comprises one or more filtering units. Each unit consists of a filter porcelain funnel AA' , similar in construction to a Seitz filter except that the two parts are clamped together by means of two metal rings held with bolts and wing nuts BB' . Soft rubber rings are interposed between the supporting grid C and the funnel portion and between the membrane and the reservoir portion of the filter.

The top of the reservoir is closed with a circular wooden cover D , the centre of which is pierced by a hole in which is held a short length (1 in.) of glass tubing. This acts as bearing for a solid glass T piece E , the side-limbs of which are inserted into two rubber sponges, FF' , cut as follows. They have cylindrical shape about $1\frac{1}{2}$ cm. in diameter and long enough to reach to within $\frac{1}{2}$ cm. of

¹ Owen Williams Research Scholar.

the central spindle and the wall of the reservoir. They are pierced longitudinally by means of a cork borer small enough to cause a tight fit on the glass arm. The sponge should be soft and loose-textured.

The T piece is connected by means of rubber tubing to a vertical shaft *G* which is made to revolve at a rate of one revolution every 3 seconds. There should be ample latitude for vertical movement of the shaft, as it is difficult to set up the filter exactly normal to the T piece. Constant pressure of the sponges

on the membrane is maintained by means of weights placed on the tray *H*. We have found that 50 g. is suitable for a 7 cm. filter, but the weight may need increasing when the residue becomes very viscous.

Suction is applied by the usual method from a good water-pump. A large reservoir is interposed between the pump and the filter battery in order to avoid extreme oscillations of pressure when connecting up a new filter while others are in use.

Several sponges are kept as spares. To clean them, they are first thoroughly rinsed in 3 % NaOH and then left for several days in 2 % Na_2CO_3 solution.

When subsequently rinsed with running tap-water and finally placed in distilled water for an hour or so they are ready for use. They should never be allowed to dry.

Preparation of the membranes.

Membranes were made with the following objects.

- (1) To keep back protein.
- (2) To have sufficient degree of surface hardness to avoid damage by the wipers.
- (3) To have sufficient strength to withstand pressures of one atmosphere.
- (4) To have a rapid filtration rate.

Two types of membranes have been found to fulfil these requirements.

(1) *Alcohol-ether-acetic acid-collodion membranes.* Pyroxylin is dried in a vacuum desiccator until of constant weight; this usually requires 3 to 4 days. From this a 6 % stock solution is made in a mixture containing 50 parts ether, 50 parts alcohol and 10 parts glacial acetic acid. After 3 days the stock can be used. We do not take particular precautions to render the ether and alcohol free from water. The ether used is Howard's anaesthetic ether and the alcohol is about 99.5 %.

The membranes are made from a solution containing 2 % pyroxylin by diluting down the stock with the mixture of alcohol, ether and acetic acid to the required strength.

The membranes are prepared on polished glass plates whose diameter is about 1 cm. greater than that of the filter. The plates are cleaned in dichromate and sulphuric acid, washed with distilled water, dried and polished with a silk cloth. The surface must be free from scratches and dust particles.

Holding the plate in the left hand about 5 cc. collodion are poured on to the centre, and by rapid tilting a thin film of collodion is formed covering the whole plate. By rapidly rotating in a vertical plane any excess of collodion is allowed to run off. The plate is now placed on mercury in a sulphuric acid desiccator. Here evaporation takes place in a still atmosphere—ensuring uniform drying of the membrane—from which moisture is precluded, thereby preventing condensation of moisture on the surface while the ether evaporates. In the absence of this latter precaution the film takes up moisture as it dries and a milky appearance or “blushing” of the membrane occurs. After a period of 5 minutes the plate is removed from the desiccator and the surface observed by reflected light. The appearance of a fine network pattern over the whole membrane surface indicates that drying is complete. The plate is now placed in distilled water. Gelling of the collodion occurs, the membrane contracts and floats off the glass in 1–2 minutes. It is then floated on to a filter-paper and placed in position in the filtering chamber, where it is washed free from acetic acid with distilled water.

These membranes are ideal for rapid filtration of plasma. They are, however, too fragile to be used repeatedly. In our experiments we found it desirable to filter plasma from patients under investigation and from control subjects through the same membrane, in order to eliminate the possibility of variable permeability of different membranes. We therefore attempted to prepare a stouter membrane impermeable to protein and suitable for successive filtrations.

(2) *Glacial acetic-collodion membranes.* The most suitable for our purpose were found to be glacial acetic-collodion membranes on a filter-paper basis similar to those described by Kreuger and Ritter [1930]. Hard Whatman (No. 52) filter-papers were immersed in collodion solution and the excess drained

away by rotation in a vertical plane. Gelling was brought about by immersion in distilled water and the excess acetic acid removed by washing in running water overnight.

Varying concentrations of pyroxylin in glacial acetic acid were tested. It was found that with concentrations of less than 4 % the membrane surface was damaged by the rotating sponges. Membranes of 4 % collodion can be used for several filtrations without deterioration of the surface. The rate of filtration under 70 cm. pressure is slightly slower than with the alcohol-ether-acetic acid-collodion membranes.

After filtration, the membrane surface is cleaned with 2 % sodium bicarbonate solution and the membrane washed on the filter with 50 cc. of the same solution followed by distilled water. It can then be preserved in distilled water until required for further use.

DISCUSSION.

We have obtained very satisfactory and consistent results using these types of membranes. Although the mode of preparation seems to lack that careful standardisation which many workers insist upon, we are convinced that the precautions taken are sufficient. Thus, for example, the exact volume of collodion used for each membrane is not measured, and one would expect varying rates of filtration from membrane to membrane owing to alteration in thickness. This does not seem to be the case, and we are of the opinion that the factor which determines the rate of filtration, and to a great extent the pore size, is the nature of the surface of the membrane. The thickness plays only a minor part. It is for this reason that the mode of drying the membrane is the most important part of the preparation to be controlled. The method is well suited for both purposes for which it was devised. For example, it is possible to obtain 25 cc. ultrafiltrate from 30 cc. plasma in 30 minutes. In certain injection experiments, we found that the time from taking the blood from the vein to the injection of it into the animal could be reduced to 45 minutes. As the whole filtration can be carried out at 0° and under sterile conditions, there is little danger of loss of labile substances through decomposition.

For large quantities it is equally suitable. The following figures give an idea of a typical filtration:

Vol. of plasma	1st hour cc.	2nd hour cc.	3rd hour cc.	4th hour cc.	5th hour cc.	6th hour cc.
450	100	85	70	60	50	25

SUMMARY.

1. An apparatus for rapid ultrafiltration and methods for preparing collodion membranes have been described with the object of removing proteins from blood, plasma and serum for the isolation of various constituents.
2. The method is suitable (i) for obtaining rapidly small quantities of ultrafiltrate for inoculation; (ii) for filtering large quantities.

REFERENCES.

- Elford (1931). *J. Path. Bact.* **34**, 4, 805.
 Kreuger and Ritter (1930). *J. Gen. Physiol.* **13**, 409.
 Pierce (1927). *J. Biol. Chem.* **75**, 795.
 Smith (1928, 1). *J. Physiol.* **65**, Proc. xii.
 — (1928, 2). *Biochem. J.* **22**, 1499.