LXXVIII. THE PREPARATION OF SEMI-PERMEABLE SAC-MEMBRANES.

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PRIOR to a series of studies on the partition of serum-calcium in the case of the lactating cow it was felt that a study of methods of preparing membranes and of their use in ultrafiltration would be advisable. A survey of the literature revealed a number of methods for the preparation of membranes but the time required for the manufacture and the manipulation of somewhat elaborate apparatus were definite obstacles. It was consequently felt that the introduction of a method which would give membranes capable of duplication by different workers and easy of preparation and manipulation would be advantageous.

Two contributions to membrane preparation which appear to have received little consideration are:

(1) Trendelenburg's method [1923] of casting membranes on the outside of test-tubes.

(2) Pierce's method [1927] of limiting the porosity of flat membranes by the use of ethylene glycol.

By combining these two methods the objective has been attained.

REAGENTS AND APPARATUS.

7 % Collodion solution. B.D.H. collodion is analysed for nitrocellulose content and diluted with an alcohol-ether solution (1:1 by weight) until it contains 7 % by weight of nitrocellulose.

Membrane solution. To the 7 % nitrocellulose solution is added the requisite amount of ethylene glycol found to be satisfactory by experimental trial.

15 % gelatin solution in water.

Gies reagent [1910]. To 1 litre of 10 % KOH solution add 25 ml. of 3 % CuSO_4 solution.

Test-tubes. A small hole is blown in the tip of each test-tube. The size of test-tube required depends on the amount of serum available for analysis.

The ultrafiltration apparatus. It was felt that the elimination of automatic valves and compressed air cylinders would be advisable. Ultrafiltration is carried out at a positive pressure of 175–185 mm. of Hg. and the maintenance of this pressure for one hour, without attention, can be achieved by the use of the pressure unit herein described (Fig. 1).

A. Manometer to register 175–220 mm. Hg.

B. A tube containing a Bunsen valve. This valve is necessary since it is difficult to obtain a pressure bulb which does not allow some air-leakage.

C. A tube containing normal saline.

D. A Winchester bottle as a reservoir of compressed air.

E. A wide-mouthed bottle which connects the pressure unit with the individual ultrafiltration units. In our investigations four ultrafiltration units are connected to this bottle.

F. Serum reservoir.

G. Membrane.

H. Ultrafiltrate tube.

I. A wide-mouthed bottle containing physiological saline. Since the vapour pressures of normal saline and ultrafiltrates are practically identical the losses by evaporation are minimised.

Fig. 1. Ultrafiltration apparatus.

Preparation of membranes. The test-tubes are coated with gelatin by dipping the stoppered tube into warm gelatin solution. The tubes are immersed to within 1 cm. of the lip, withdrawn and suspended in a special rack to drain and dry for several hours. The small hole in the tip should be sealed by a film of gelatin. Once dry these tubes can be stored until required.

To prepare the membrane the gelatined tube is dipped into the collodion-ethylene glycol solution to the required depth and slowly withdrawn. The tip of the tube is left in contact with the surface of the solution for a few seconds to facilitate draining and the tube is then rapidly transferred to the special rack and suspended. When removing the tube it is advisable to guard against drops of collodion falling into the solution, since this causes air-bubbles which will be picked up by subsequent tubes and cause faults in the membranes. The strand of nitrocellulose which forms at the tip of the tube as a result of draining is cut off as it forms, since the weight of it draws off too much nitrocellulose and results in fragile membranes. The entire preparation of the membrane should take about 20 seconds.

After the membranes have been drying in the rack for 20 minutes (exactly) the tube is filled with water and placed in a beaker of distilled water. The mem-

brane should be entirely below the surface. After 1 hour or so the tubes are filled with warm water and shaken vigorously to dissolve the gelatin sealing the small hole at the tip of the tube and also to melt the gelatin film between the membrane and the glass. When the membrane slides free it is removed and filled with distilled water.

Instead of tying the membranes on to rubber stoppers it has been found more convenient to tie them on to pieces of rubber pressure tubing 1.5 cm. in length. When used in the ultrafiltration unit the glass tube connecting the membrane to the serum reservoir is somewhat larger than is generally used with this size of rubber tubing. The extra distension of the rubber is enough to cause the membrane to grip the tubing quite securely. This method was found to be so satisfactory that the connections of the serum reservoir are also made from rubber tubing instead of stoppers.

After being tied on to the rubber tubing the membrane is washed free from gelatin by shaking several times with distilled water. Finally the membrane is filled with distilled water and connected to the serum reservoir, which is then filled with distilled water, and 5 ml. of water are forced through the membrane at a pressure of 220 mm. of Hg. This removes the ethylene glycol and detects faulty membranes. It is advisable not to let the level of the water fall as low as the sac since the drying of any portion of the membrane will result in fragility at that point. When the membrane is removed it is filled to the top with distilled water and closed with a short glass rod. The membranes are stored in chloroform water until required.

Determination of ethylene glycol-nitrocellulose ratio. The proportion of ethylene glycol to nitrocellulose necessary to give a sac-membrane impermeable to serum proteins is slightly variable, depending on the sample of collodion.

To a series of tubes each containing a known amount of the 7 % nitrocellulose solution are added increasing amounts of ethylene glycol. The range employed is 1.5–2.0 ml. of ethylene glycol to 1 g. of nitrocellulose. Membranes are cast by the method described above and tested by ultrafiltering a sample of serum. Such membranes as show a negative biuret test when Gies reagent is added correspond to the maximum dilution permissible. It is advisable to keep somewhat below this.

So far it has been found that a proportion of 1.8 ml. of ethylene glycol to 1.0 g. nitrocellulose (*i.e.* 12.6 ml. of ethylene glycol to 100 ml. of the diluted collodion) gives uniformly biuret-negative filtrates.

Ultrafiltration method. When required for analytical purposes the membrane is rinsed out three times with serum, the washings being removed by means of a pipette, and filled with serum. The sac is then connected to the reservoir and the reservoir filled with serum. The pressure is raised to 180 mm. of Hg. and the outside of the sac dried with filter-paper. The ultrafiltrate appears almost immediately. After about 10 minutes the pressure is reduced to about 50 mm. (sufficient to keep the sac distended to facilitate handling) and the sac dried. The ultrafiltrate tube is then connected over the sac and the pressure raised to 185 mm. The pressure falls very gradually, usually to about 175 mm. in 1 hour.

RESULTS.

It is unnecessary to quote results regarding permeability. Sac-membranes made by this method were found to be completely permeable to aqueous solutions of CaCl_2 with a calcium content of 10 mg. per 100 ml.

The main point under investigation was that of duplication. Consequently three membranes each were made by four members of this Institute and used to prepare ultrafiltrates from a sample of serum obtained from slaughter-house blood. Each ultrafiltrate was analysed in triplicate. The results are shown in full in Table I.

Table I.

Results in mg. Ca per 100 ml. of ultrafiltrate.

Made by	Membrane number	Analysis			Average
		1	2	3	
A	1	6.0	6.0	6.1	6.0
	2	6.1	6.0	6.0	6.0
	3	5.9	6.1	6.0	6.0
B	1	5.8	5.9	5.8	5.8
	2	5.9	—	5.9	5.9
	3	5.9	6.0	5.9	5.9
C	1	5.8	5.9	5.9	5.9
	2	5.9	6.0	5.8	5.9
	3	6.0	5.9	—	6.0
D	1	6.0	5.9	6.0	6.0
	2	5.7	5.9	5.8	5.8
	3	5.8	5.9	6.0	5.9

The calcium estimations were by the method of Clark and Collip [1925]. The experimental error of this method is $\pm 2\%$. A study of the results in Table I indicates that the variation in the calcium contents of the various ultrafiltrates was of this order. It would appear, therefore, that membranes made by the proposed method, and by different investigators, will yield identical results.

It is a point of interest that the membranes used in this experiment were two days old, with the exception of those prepared by D, which were over a week old. The results do not appear to be affected by the age of the membrane, at least within this limit. Also the membranes used were those prepared for the first time by all workers except D.

SUMMARY.

A method of preparing sac-membranes is described which will yield practically identical ultrafiltrates, at least in so far as the calcium is concerned, at the hands of different investigators.

REFERENCES.

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