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Technical Methods

Simple ultrafiltration apparatus

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All ultrafiltration devices have in common a semi-permeable membrane and a means for creating a pressure difference across it. They differ only in the configuration of the membrane, the nature of the force used to create a pressure difference, and the ease with which the environment of the plasma can be controlled so as to simulate physiological conditions. Speed of producing ultrafiltrate provides a poor criterion of performance since most devices can be sealed up to give any desired flow rate. The limiting factor in practice is usually sample size.

The apparatus described here permits the ultrafiltration of volumes of plasma down to about 1·5 ml. with the formation of ultrafiltrate equal to half the volume of plasma within 15 min. under controlled conditions of temperature and *p*H. The principle of the apparatus is as follows. Plasma is separated anaerobically and transferred to an all-glass syringe from which it is forced under a pressure of 4 atmospheres into a cellophane sac. The sac is invaginated in order to provide a high ratio of surface area to capacity and is supported on the outside by a spiral of stainless steel to prevent rupture.

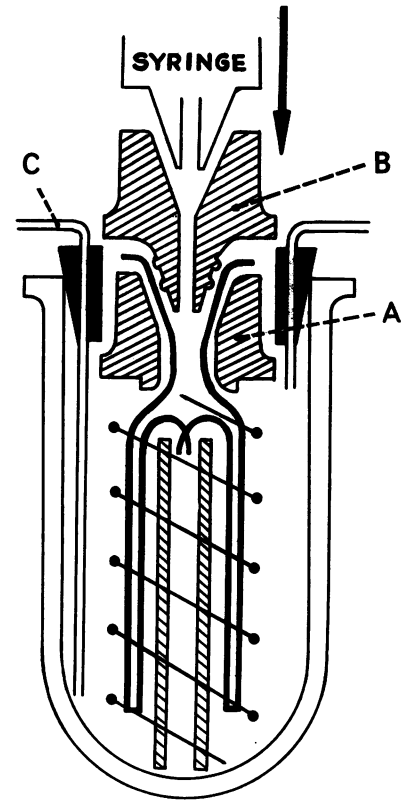

FIG. 1

CONSTRUCTION

A nylon female flanged Luer adaptor[1] (A, Fig. 1) has all but a small shoulder of its tapered shank cut off, the sharp edges carefully smoothed and the body mounted in the plastic stopper of a disposable polythene 90 × 16 mm. tube. A length of ¼ in. Visking tubing[2] is knotted tightly at one end and the other end folded longitudinally and carefully pulled through A until there is 15 cm. tubing between the lower end of A and the knot. The tubing is then cut off just above the upper end of A, the lumen opened with forceps and a tight seal established by placing the tapered end of another adaptor, B, within the tubing and forcing it hard into the Luer entry of A. This operation requires either a strong grip or the use of tools made up to grip the adaptors.

A syringe filled with distilled water can now be inserted into B and the Visking sac gently distended. The knot is inserted into a length of approximately 6 mm.O.D. glass tubing which is used to invaginate the sac. The water is withdrawn by suction on the syringe which is then disconnected. A stainless steel spiral (prepared by winding a length of 1·0 mm. diameter hard stainless steel wire[3]

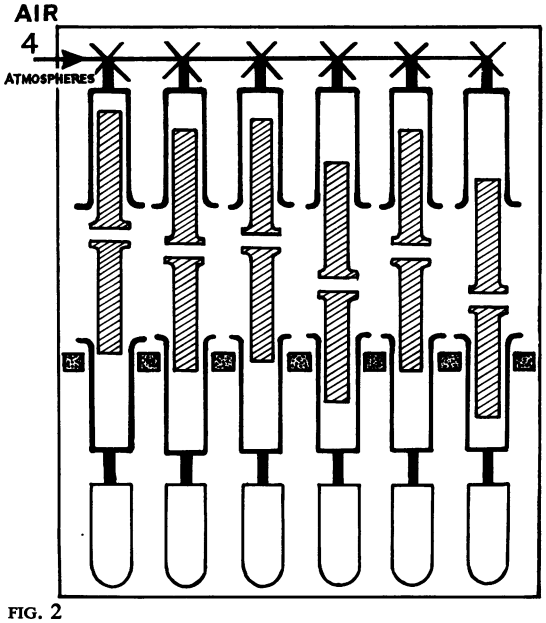

FIG. 2

[1]Portland Plastics Ltd., Hythe Kent. A, M722 (7/- doz), B, M700 (6/6 doz).
[2]The Scientific Instrument Centre Ltd., 1 Leeke St., London, W.C.1. 50/- per roll = 120 sacs.
[3]K. C. Smith & Co., Redbrook Rd., Monmouth, Mon. 1 lb. 1·0 mm. hard wire is sufficient for 100 spirals. (14/5 lb.).

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on a ¼ in. mandrel and pulling out to a pitch of 3·5 turns per cm.) is passed over the invaginated sac and the whole inserted into the 90 × 16 mm. polythene tube. The diameter of the glass tube is selected so that the sac covering it makes an easy sliding fit within the steel spiral.

Needles, C, are inserted through the stopper to allow passage of gas mixtures and collection of sequential samples of ultrafiltrate.

The apparatus can be stored in this moist state indefinitely. It is prepared for use by drying at 37°C. for one hour or by rinsing with the plasma to be filtered.

OPERATION

A 10 ml. Chance metal tip interchangeable syringe with centre nozzle is lubricated with sufficient stopcock grease to prevent backflow under pressure. The syringe is filled with the plasma to be ultrafiltered and the nozzle inserted into B. Pressure can be applied, with the syringe held upright in a firm clamp, by placing a 15 lb. lead weight on the plunger. With the syringe described, this weight exerts a pressure of 4 atmospheres within the sac. A less cumbersome arrangement is to construct an inclined stand in which six syringes can be supported with their ultrafiltration tubes. Pressure is applied to each syringe by an identical syringe mounted in an inverted position above the first and fed with air at 4 atmospheres (Fig. 2).

The sac as described has a volume of 1·5 ml. It will form 1·5 ml. ultrafiltrate from 3 ml. plasma in 15 min. at 37°C. With the steel spiral described, most sacs would burst at a pressure of about 6 atmospheres; at 4 atmospheres, none has burst in more than 200 operations. Failure may rarely occur from tearing due to excessive torsion at the union of A and B or from inadequate smoothing either of the cut end of A or of the ends of the steel spiral. No failures have occurred except at the moment of initial application of pressure, and at this stage it is fairly easy to transfer the sample to a fresh sac. The weakest point of the sac is the reflection at the lower end of the invagination. An excessively coarse pitch of the steel spiral is to be avoided in this region although alarming bulging of the sac here is readily supported by the inner surface of the polythene tube.

The apparatus as described can be used with plasma containing radioactive tracers without hazard. If radioactive material is not present, the construction of an individual sac may be simplified by filling a syringe with plasma and inserting its tip directly into A. The Visking tube is then distended with the plasma, invagination is carried out and the device assembled without detaching the syringe. For single samples, this procedure is quite convenient and does have the advantage of eliminating the dead space of B; this may be useful if plasma volume is limited.

COMMENT

Previous ultrafiltration devices have been reviewed by Ames and Sakanoue (1964). The apparatus they describe also uses a syringe to transfer plasma under pressure into a sac but it requires a minimum volume of 8 ml. plasma. Devices using centrifugal force to provide a pressure difference (Toribara, Terepka, and Dewey, 1957) can function with 3 ml. plasma but require several hours to filter 1 ml. The present device achieves very rapid ultrafiltration by creating a large ratio of surface area to sac volume as a result of invaginating a Visking tube rather like a renal glomerular capsule. High pressures can be used because of the support afforded by the steel spiral. The temperature and gaseous surroundings of the sac can readily be controlled so that a physiological environment can be simulated.

SUMMARY

A device for plasma ultrafiltration is described that is simple, flexible, rapid, cheap, robust and reliable.

I wish to thank Dr. G. K. McGowan for his advice and encouragement.

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Ames, A., III and Sakanoue, M. (1964). *J. lab. clin. Med.*, **64**, 168.
Toribara, T. Y., Terepka, A. R., and Dewey, P. A. (1957). *J. clin. Invest.*, **36**, 738.