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The Dialysis of Small Volumes of Fluid

By N. G. HEATLEY

(*The Sir William Dunn School of Pathology, University of Oxford*)

A simple, non-metallic closure for small dialysis tubing is described. In a smaller-scale procedure the 5 to 50 μl of solution to be dialysed are placed on a circle of cellophane, 7·5 mm in diameter, held by suction to the upper end of a vertical tube containing water or saline, which may or may not be continuously renewed. By enclosing the tube, disc and drop in a chamber containing desiccant, the fluid on the disc is concentrated and this greatly accelerates dialysis. A modification for quantitative collection of the whole of the diffusible material in a small volume of fluid is described. Performance data on solutions of sodium chloride are given. The apparatus is well suited to multiple operation.

The routine dialysis of volumes of fluid down to a few tenths of a millilitre is almost universally carried out in simple knotted sacs of the smallest size of dialysis tubing. Special methods have also been described,[1,2,3,4] but there are no standard methods of dialysing much smaller volumes. The present paper describes two modifications of a simple procedure for dialysing from 5 to 50 μl of fluid, the first for removing unwanted diffusible material, and the second for when the diffusate is needed also. This is preceded by a description of a simple closure for dialysis tubing, which has been used for some years.

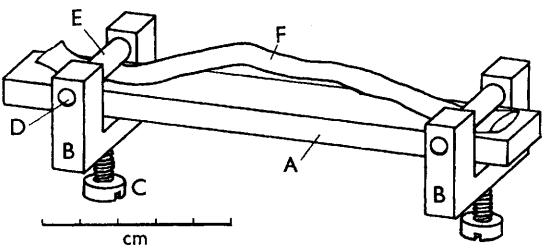

Fig. 1. Bar and clamps for closing dialysis tubing. Lettered parts of the diagram are referred to in the text

Non-metallic clamps for dialysis tubing (fig. 1)

This system has been used for some years with the smallest size of dialysis tubing. Instead of being knotted, the ends of the tubing are closed by clamping against a rectangular Perspex bar, A, the whole being suitably rocked, rotated or agitated in the required water or salt solution. The dimensions of the clamps B are unimportant, nor is accurate machining required. The body is of Perspex. D is a length of glass rod fitting loosely into holes in each arm of the body, and held in place by the sleeve, E, of rubber tubing. This rubber sleeve also provides a cushion against which the flat, wetted dialysis tubing, F, is pressed by the bar, A, when the nylon screw, C, is tightened. Gentle finger tightening of C gives a closure, completely leak-proof up to the bursting pressure of the dialysis tubing (about 20 p.s.i.).

The advantages are that the sac is easily opened for removal or addition of material; it can be re-used; the contents can be removed more completely than from a knotted sac; and the risks associated with knots (leakage and stretching of membrane) are avoided.

Micro method for the removal of unwanted diffusible material

Principle—

The apparatus is shown in Fig. 2. The upper end of glass tube A, containing water, is closed by a cellophane disc, B, which is held in place by the suction resulting from the reservoir, C, being about 20 cm below the disc. This same suction draws water through the fine polythene tube, D, from the main reservoir, L, so that the underside of the disc, on which is placed the solution to be dialysed, is swept with a continuous slow stream of water.

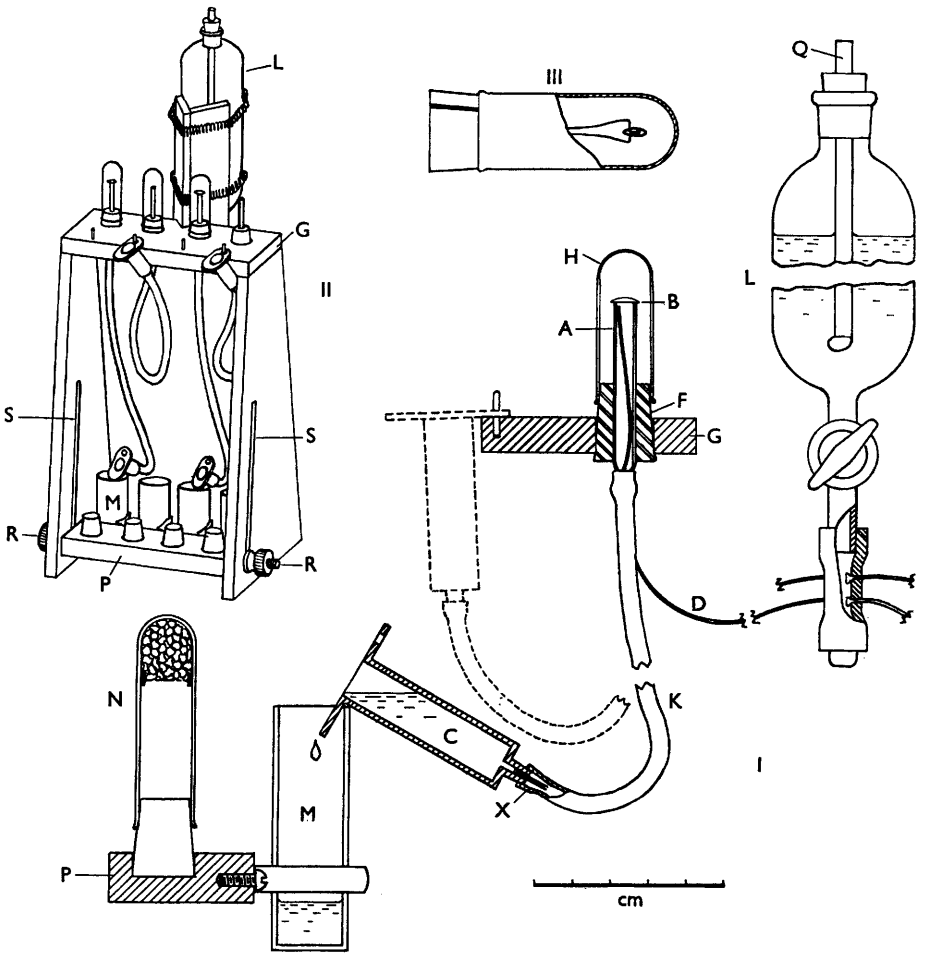

Fig. 2. Dialysis apparatus for removal of unwanted diffusible material. Lettered parts of the diagram are referred to in the text

I. A single dialysis unit
II. Semi-perspective view of stand with four units
Scale, 0·4 × that of I
III. Arrangement for supporting disc in bacterial transfer experiments

Construction—

Tube A is 5·0 mm in external diameter and thin-walled. Its upper end is ground flat with fine carborundum powder and flame-polished. It is held in a rubber bung, F (15 mm diameter at the smaller end), which in turn is mounted with several similar units in bar, G, of the Perspex or wooden stand (Fig. 2, II). (For clarity a stand with only four units is shown.) The reservoir, C, which can be the barrel of a 2-ml plastic syringe, is connected to A by the rubber tube, K, in which a short piece of plastic or glass capillary tubing, X, has been inserted

to act as a brake and give smooth control of the water level in A when C is raised or lowered. The polythene tube, D, has a bore of 0·28 mm, and when 25 cm long will permit a flow of 2 to 3 ml hour^{-1} under a pressure difference of 20 cm of water. It is inserted obliquely[5] through the wall of tube K.

All the units are fed from one main reservoir, L, through a manifold.[5] Initially, a polythene spider at the top of A kept the end of D co-axial with it, but this was shown to be unnecessary. When a unit is not in use, the reservoir, C, is hung from a pin in G (broken lines in Fig. 2, I), but in its working position it hooks on and empties into a container, M, held in a Terry clip at the back of bar P. This enables the volume of rinsing water passing through each unit to be checked. Bar P adds rigidity to the frame and can be fixed at different heights by the knurled nuts, R, on bolts passing through grooves, S. Tube A, with its disc and fluid to be dialysed is normally protected by a glass cap, H, the rim of which rests on the bung, F, a groove in the latter preventing changes in pressure when H is put on or removed. One or more additional sets of caps containing indicating silica gel are also required (Fig. 2, N); the disc of metal gauze, which retains the desiccant, is kept in place by two expanding metal rings. The desiccant in these "drying caps" is regenerated by baking the cap as a whole. After baking they are closed by rubber bungs, *e.g.*, by the four bungs on bar P, which provide stands for the drying caps when not in use.

Reservoir L, held by springs in a vertical trough fixed to the frame, is closed by a bung carrying an air-inlet tube, Q, the lower end of which is level with or slightly below the discs; thus the tubes, D, do not empty, nor does water overflow when no disc is in place.

CELLOPHANE DISCS—

These are cut with a sharp cork-borer, 7·5 mm diameter, from several thicknesses of wet cellophane, and are stored, after washing, in 50 per cent. ethanol to prevent microbial attack. If discs are cut from flat dialysis tubing it is essential that it is first wetted and distended, otherwise it is difficult to separate the pairs of discs. A disc of cellophane, 0·02 mm thick in the dry state, will easily support a negative pressure of 50 cm of water without buckling when set up as described. Other membranes have so far not been used.

MANIPULATION—

A cellophane disc is picked up with forceps and rinsed with water. Reservoir, C, is raised until the water in tube, A, just protrudes, and the disc is placed on it. C is lowered 2 to 3 cm, excess fluid under the disc outside A is removed by lifting one side of it, the disc is centred, and C is hooked on to the collection vessel, M. Any water on the top of the disc is removed with a narrow thread of filter-paper or a pipette, and the solution to be dialysed is placed on the disc. Though a particularly steady hand is not required, rough handling of the disc will allow air to enter A. This risk can be avoided by handling fluid on top of the disc with a polythene pipette that has a fine and flexible tip.

The water or solution in the reservoir, L, should be partly de-gassed by brief exposure to vacuum or by boiling, otherwise bubbles may form under the disc. Small bubbles, however, have little effect on the rate of dialysis.

IS THERE LEAKAGE AROUND THE EDGE OF THE DISC?

A sterile disc was supported between two stainless-steel loops (Fig. 2, III) and 17 to 27 μl of sterile broth were placed on one side of it and the same volume of a broth culture of a highly mobile Proteus on the other. After standing for 1 to 2 hours, with the culture on top, both drops were plated-out on well dried nutrient agar, the top drop after suitable dilution, and were counted. In three of fifteen such experiments the lower drop remained sterile; in the remainder the leakage was such that for every cell in the lower drop there were at least one million (often many millions) in the upper.

Possible leakage from large drops was tested by placing a disc on the tube of the modified apparatus (Fig. 5) over a column of water. A small volume of water was then placed on the under surface of the disc outside the tube A. A 100-μl volume of a 1 per cent. solution of the non-diffusible dye Pontamine Sky blue 6BX was placed on the disc and covered with a cap. After 1 hour the fluid on the underside of the disc outside A was sampled with a strip of filter-paper or a pipette and examined for blue colour. This test showed that even with 100 μl on the disc there was no leakage around the edge. A drop of this size is, however, extremely unsteady and leakage does occur if it is not handled carefully.

FACTORS AFFECTING DROP SIZE—

As the disc is under suction, there is a slow passage of water through it, of about 0·02 μl hour^{-1} cm^{-1} water suction. The osmotic swelling with concentrated solutions is, however, considerable (Fig. 3), and with substances less readily diffusible than sodium chloride will presumably be greater. It must be taken into account in deciding how large a drop can safely be placed on the cellophane disc. As diffusible materials leave the drop osmotic swelling will decrease and then cease, but in high concentrations of non-diffusible substances there will be a continuous swelling. Osmotic swelling can be countered by the use of the drying caps, as shown by the broken lines of Fig. 3.

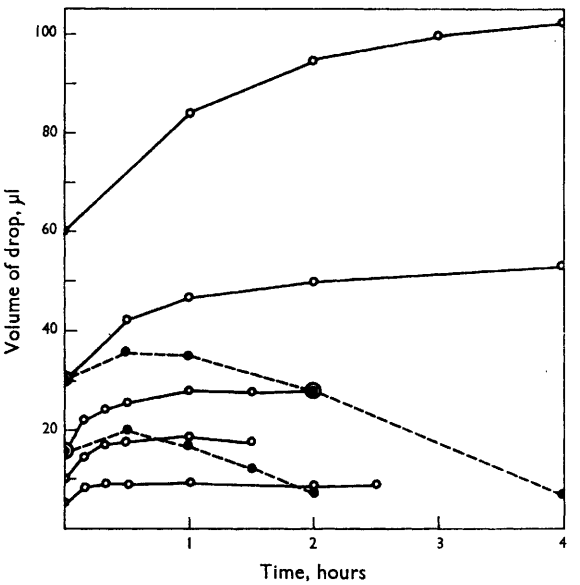

Fig. 3. Osmotic swelling of drops of different initial volumes of 5 M sodium chloride during dialysis in apparatus shown in Fig. 2: with drying caps – – ● – – ● – –; and without drying caps —○—○—

VOLUME OF FLUID LEFT ON DISC—

By forming a drop of Pontamine Sky blue on a disc, removing the drop in the usual manner, then vigorously agitating the disc in a known volume of water for 10 seconds and measuring the dye spectrophotometrically, it was shown that about 0·5 μl of solution was left on the top of the disc. This "dead space" volume was disregarded in calculating the results of Figs. 3 and 4, but it might be of importance when small volumes are being dialysed.

EFFECT OF FLOW-RATE OF RINSING WATER ON RATE OF DIALYSIS—

With drops of 5 or 10 μl of 5 M sodium chloride the rate of dialysis was almost the same when, by accident, the tap of the main reservoir was not opened, as it was under the normal flow (under a pressure difference of 20 cm of water); no doubt the dense solution diffusing through the disc falls far into tube K. However, it was felt that with larger drops of more dilute solute the flow-rate might affect the rate of dialysis. This was tested with drops of 30 μl of M sodium chloride, left to dialyse for 1 hour under the normal rinsing water pressure difference of 20 cm, and also under 10 and 5 cm. The rates of removal of solute were virtually identical. Thus, with sodium chloride the flow-rate of rinsing water is not a limiting factor even down to one quarter of the normal flow-rate; with less diffusible substances it would be even less so.

RATE OF REMOVAL OF SODIUM CHLORIDE FROM DROPS OF DIFFERENT INITIAL SIZE, WITH AND WITHOUT DRYING CAPS—

A series of conductimetric measurements of the percentage of sodium chloride (initially 5·0 M) left in drops of different initial size after different periods of dialysis is summarised in Fig. 4. The rate of dialysis predictably becomes lower as initial drop size increases, but with drops of any given initial size the use of drying caps progressively accelerates the rate of dialysis so that the longer dialysis proceeds the greater their effect. Thus, an initial volume of 30 μl of 5 M sodium chloride has, after 2 hours, half as much solute left with drying caps as with plain caps; after 4 hours this ratio has dropped to 1:20.

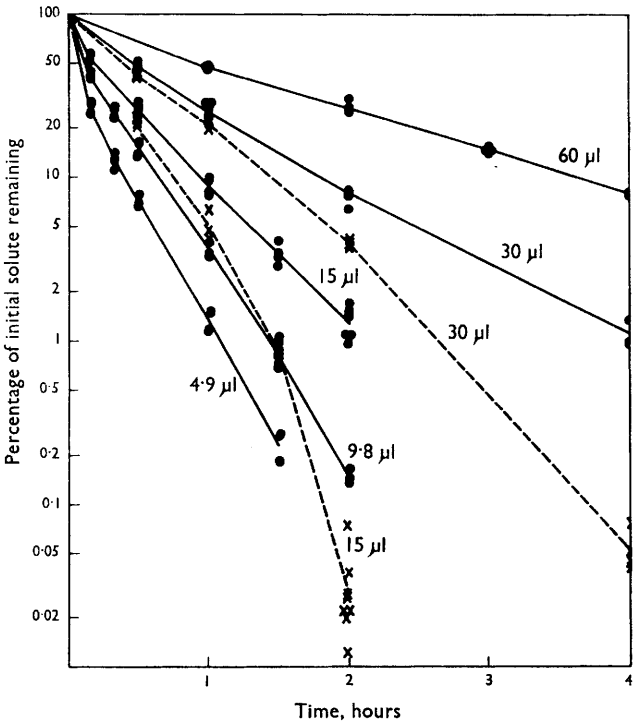

Fig. 4. Course of removal of solute from drops of 5·0 M sodium chloride of different initial size, after different periods of dialysis in the apparatus shown in Fig. 2. Figures beside curves show initial drop size, μl: with drying caps – – X – – X – –; and without drying caps —●—●—

EFFICIENCY OF DRYING CAPS—

The rate of uptake of water vapour by the silica gel appears to be largely independent of the particle size of the gel, but it varies inversely with the distance of the gel from the drop. That diffusion into the gel is not a limiting factor is suggested by the fact that partial exhaustion of the gel is indicated by a fairly sharply defined zone of colour change. As the effective part of the gel moves further from the drop, the rate of transfer of water will become correspondingly slower. With a freshly baked cap, with the metal mesh about 8 mm above disc B, the rate of removal of water will be about 10 μl hour^{-1}.

MODIFICATION OF MICRO APPARATUS WHEN BOTH DIFFUSATE AND RETENTATE ARE NEEDED

PRINCIPLE—

A cellophane disc is held by suction on the top of a tube that has been made water-repellent by treatment with a silicone. The fluid to be dialysed rests on the disc, as before, the rinsing fluid being a short column of water immediately below the disc. Its position in the tube

is controlled by compressing a rubber sac containing air. Thus, to place a disc in position, the column of water is made just to protrude from the top of A, the disc is laid on it, centred, and then held in position by creating slight suction.

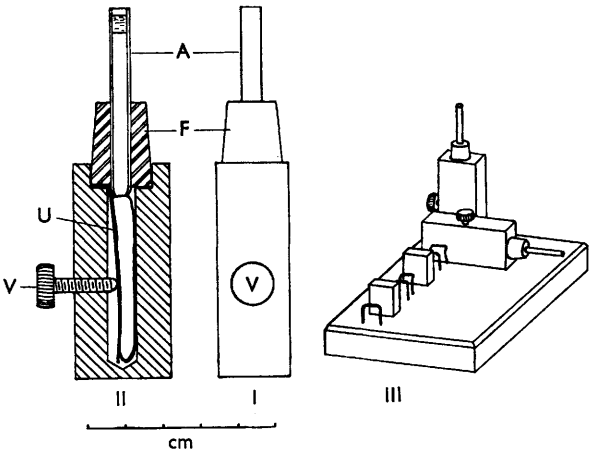

Fig. 5. Micro-dialysis apparatus. Modification for when diffusate is required as well as retentate. Lettered parts are referred to in the text

I. Side elevation
II. Median vertical section
III. Projection of stand for holding six units (III is 0·4 × scale of I and II)

CONSTRUCTION—

Fig. 5 shows one of many possible designs. The body of the unit is a simple rectangular Perspex or wooden block in which rubber bung, F, carrying tube A is mounted. The rubber sac (a fountain-pen reservoir) on the lower end of A is compressed by the bar, U, controlled by screw, V. Six such units are held between spring clips and blocks on a heavy base (Fig. 5, III), tube A being vertical or horizontal, as required; the unit can be removed if, for example, fluid in A is to be discharged into another vessel. The base is not essential, but enables screw, V, to be operated easily with one hand.

MANIPULATION—

With tube A horizontal, a column of water, of known volume if desired, is placed in it. The unit is turned so that A is vertical, and a disc is placed in position as described. At the end of dialysis the drop is removed with a pipette and the disc is drawn off horizontally with forceps; its under-surface will have a small volume of fluid adherent, but this can be removed in a pipette and added to the main bulk of the diffusate. For removal of the latter, tube A is again made horizontal and the diffusate removed with a pipette. Or the unit can be inverted over a receiving vessel and the fluid expelled by turning screw V.

USE OF DRYING CAPS—

As with the first apparatus (Fig. 2) reduction of the volume of the drop on the disc by means of a drying cap will accelerate dialysis. Also by reducing the volume of the retentate in relation to the diffusate, more of the total amount of diffusible material can be made to pass into the latter.

DIALYSIS OF THE SAME DROP AGAINST SUCCESSIVE VOLUMES OF FLUID—

Provided the drop is not too large, or has been concentrated sufficiently by a drying cap, it is a simple matter to dialyse the same solution against successive volumes of water; another unit is prepared with the water just protruding from the tube, and the disc is drawn off horizontally with fine forceps and placed on this second column of water in the usual way. The successive columns of water can even be in the same unit; the disc is drawn off and

placed on a siliconed surface, the tube is turned to the horizontal position, the existing column is drawn back by screw V, and a fresh column is formed on which the disc is mounted, and so on. The minimum volume of a water column that can be easily manipulated is about 10 μl, but as a column will not form with so small a volume, sufficient fluid is transferred by pipette into the tube, a column is formed and all but the required 10 μl removed.

Successive transfer to fresh water in this way, coupled with the use of drying caps, enables nearly all of the diffusible material to be recovered, and in a small volume of diffusate. One example will illustrate this: with 10 μl of 5·00 M sodium chloride on the disc and a column of 100 μl of water below, equilibrium was substantially obtained after 2 hours when the drop size was 16 μl and 85 per cent. of the total sodium chloride had been transferred to the 94 μl of fluid below the disc. But when the same column of sodium chloride was dialysed with drying caps against three successive volumes of 20 μl of water, each for 20 minutes (*i.e.*, for a total of 1 hour), nearly all of the solute was transferred to the combined water layers, whose volume was about 54 μl.

It might in some instances be necessary to add water or saline to the material on the discs, especially if it has become dry or nearly dry.

Discussion

Both forms of micro apparatus are easy and quick to set up and are well adapted to multiple operation. Fortunately, gravity exerts a doubly favourable effect with dense solutions, such as concentrated ammonium sulphate solution, for in the upper phase it will bring the more dense part of the fluid to the surface of the membrane, while below the membrane dense fluid will sink away.

Concentration of the fluid on the disc by means of the caps containing desiccant enables osmotic swelling to be controlled, and concentration may, *per se*, be useful. More important, reduction of the drop size greatly increases the rate of dialysis and also enables large drops, which might otherwise require an unacceptably long period of dialysis, to be satisfactorily treated. Still larger volumes can be processed by adding successive portions to the drop on the disc as its volume decreases.

The drying caps are also useful with the second modification, when the diffusible material is needed. If transfer to successive portions of lower phase is also effected the diffusible material can be collected quantitatively in a small total volume of diffusate.

Quantitative results for the micro methods described have been obtained for sodium chloride and for one kind of membrane only, but information obtained on a macro scale for the behaviour of other solutes with the same or a different membrane should be more or less directly applicable to the micro methods. The concentrating and accelerating effects of the drying caps will be relatively greater with substances less diffusible than sodium chloride.

Reduction of the volume of the solution being dialysed with a drying cap is akin to pressure ultrafiltration with the advantage, sometimes important, that the non-diffusible component will remain more evenly dispersed and will not concentrate at the surface of the membrane.

Commercial ultrafiltration membranes of graded porosity can probably be used in the procedures described as they are considerably more rigid than cellophane. Indeed, it may be possible to increase suction to a point at which ultrafiltration becomes significant. But complete separation of diffusible and non-diffusible solutes should be possible by multi-stage dialysis. In view of the high cost of these membranes, the small size of the disc should be an advantage.

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