Equilibrium Dialysis

N. BUCHANAN, C. EYBERG

SUMMARY

A technique of equilibrium dialysis with the use of a recently available dialyser is described.

S. Afr. Med. J., **48**, 1867 (1974).

The majority of drugs combine to a greater or lesser extent with plasma albumin;[1] the bound fraction of the drug is pharmacologically inactive, while the unbound (free) fraction is active. Hypo-albuminaemia, for example, as in the nephrotic syndrome[2] or miliary tuberculosis,[3] increases the amount of free drug available. This principle could be of therapeutic and economic importance in the context of antimicrobial therapy in malnutrition.

Dialysis has in the past presented technical problems, being complicated and time-consuming. A new dialysis system based on the equilibrium technique has now become available, and it is the principles and practical application of this apparatus (Kontron Diapack; Kontron, Zurich) that we wish to report.

PRINCIPLES OF EQUILIBRIUM DIALYSIS WITH THIS APPARATUS

Two half cells are separated by a semipermeable membrane of regenerated cellulose (0,025 mm thick) which is said to retain compounds with molecular weights between 10 000 and 20 000. In one half cell is inserted serum containing the drug being studied in the form of a drug protein complex, i.e. a macromolecule. On the other side is inserted saline in equal volume. The two solutions are brought into contact by rotation, which spreads the fluids over the surface of the membrane, providing a considerably larger surface area for equilibration.

At equilibrium, the serum and saline are recovered and analysed for the drug in question. At the outset of the procedure, the serum side contains all the drug, both bound and free, while the saline side contains none. At equilibrium, the bound fraction has remained unchanged, and the free fraction has equilibrated across the membrane. On the serum side there is bound and free drug, while on the saline side there should be solely free drug, assuming the membrane characteristics for albumin retention to be those claimed by the manufacturers. Thus the free and bound fractions can be derived by simple subtraction.

THE APPARATUS

The apparatus consists of a series of half cells made of Teflon (Fig. 1) which have a centrally depressed area. Two holes exist in each half cell, and run from the perimeter to the central depression. These are used for introducing and removing fluids. In Fig. 1, metal plugs can be seen in these holes.

A membrane, previously soaked in saline, is placed across 1 half cell (Fig. 2), and the 2 half cells are interposed, forming a single 'functional cell unit' (Fig. 3). Each cell unit is then mounted between spring-loaded cell connections in a cell block. When the 5 cells which make up any one cell block have been inserted, they are held in place by an upper Teflon flange which is screwed into place (Fig. 4). The cell block is then mounted into the rotational drive unit, as shown in Fig. 5.

Metabolic and Nutrition Unit, University of the Witwatersrand and Department of Paediatrics, Baragwanath Hospital, Johannesburg

N. BUCHANAN, B.SC., M.B. B.S., M.R.C.S., L.R.C.P., F.C.P. (S.A.)
C. EYBERG, B.SC.

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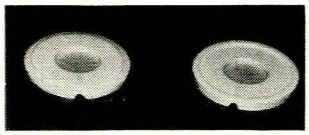

Fig. 1. Two half cells. The centrally depressed areas into which fluids are inserted are clearly visible.

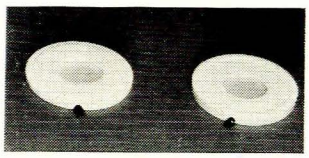

Fig. 2. A membrane is placed on one of the two half cells.

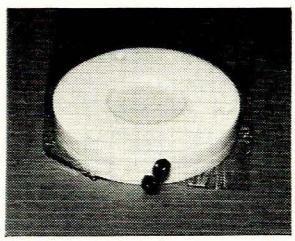

Fig. 3. A single 'functional' cell unit.

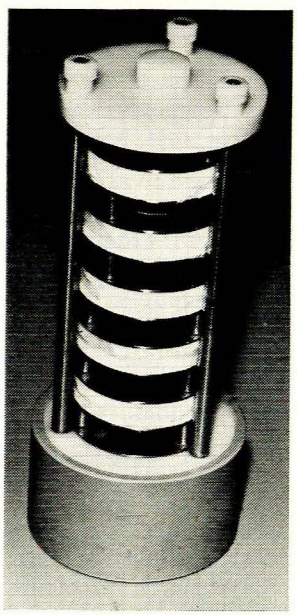

Fig. 4. Five cell units placed in the cell block.

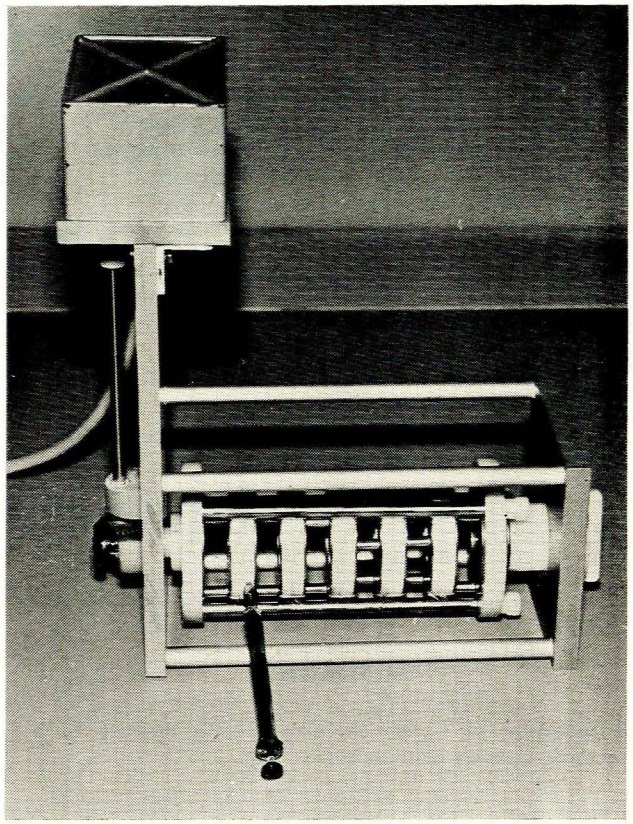

Fig. 5. The completed apparatus.

The half cells are now ready to be loaded with whatever fluids are being used. This is done by syringe, and the holes are then plugged with metal stoppers. The drive unit is placed in a water bath at 37°C, and rotation is allowed to occur until equilibrium is reached.

The particular apparatus used in this study is of the semi-micro variety with a total half cell volume of 1,36 ml and a membrane area of 4,52 cm^2.

PRACTICAL APPLICATION

Two principles needed to be established before acceptance of this apparatus as satisfactory for the studies which we had in mind: (*i*) that the dialysate was indeed albumin-free; and (*ii*) the rapidity of equilibration for the drug under study, namely, salicylate.

Fig. 6 shows an electrophoretic strip of pre- and post-dialysis specimens of fresh serum. It can be seen that albumin is essentially absent after dialysis.

Using albumin antiserum for albumin estimation, the electrophoretic findings were confirmed (Table I). Thus it appears that only about 0,2% of the albumin crosses the membrane. The dialysate can thus be considered essentially albumin-free and hence any drug present in the dialysate could be assumed to be 'free'.

With respect to equilibration time, we initially allowed 6 hours. It appeared that equilibration occurred after 60 minutes in the case of salicylate, the small subsequent

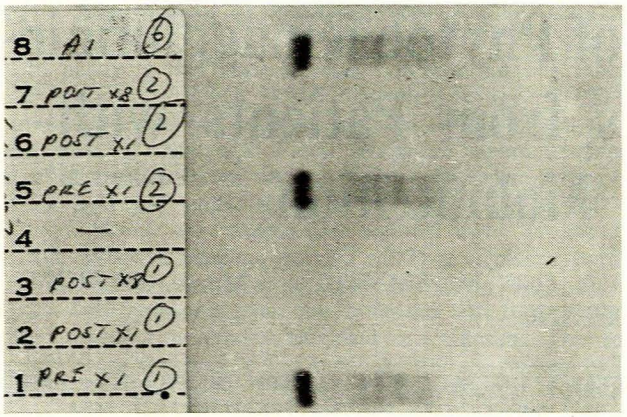

Fig. 6. Lines 1 and 5 shows the electrophoretic pattern of the serum used in these experiments. Lines 2, 3, 6 and 7 are electrophoretic strips of the dialysate.

TABLE I. RESULTS OF IMMUNODIFFUSION STUDIES WITH ALBUMIN ANTISERUM ON POOLED PLASMA AND ITS SUBSEQUENT DIALYSATE

Albumin concentrations	
Predialysis	Postdialysis
3,1 g/100 ml	**9,2 mg/100 ml**
	11 mg/100 ml
	4 mg/100 ml
3,5 g/100 ml	**5,4 mg/100 ml**
	14 mg/100 ml
	0 mg/100 ml

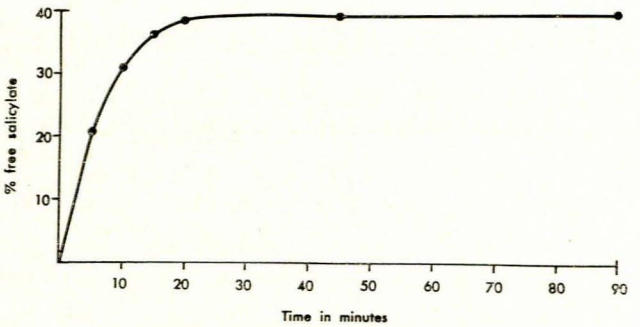

Fig. 7. Equilibration over a 90-minute period estimating one half cell only for salicylate.

variations being experimental error. This experiment was repeated over a 90-minute period (Fig. 7); it can be seen that equilibration was completed after 30 minutes.

Thus the criteria which we sought, namely those of albumin-free dialysate and rapid dialysis, appear to have been met by this apparatus. *In vitro* studies with salicylate are currently in progress and will be reported upon at a later date.

We should like to thank Dr T. Ipp of the South African Institute for Medical Research for the electrophoretic studies.

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