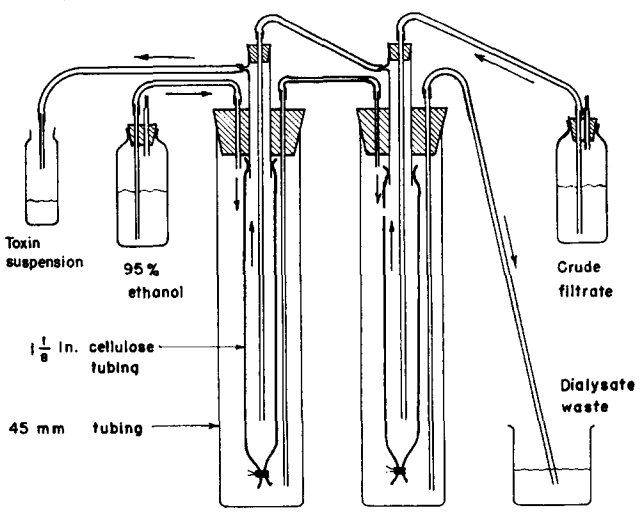
An apparatus for continuous counter-current dialysis

During studies on the purification of an extracellular toxin from *Pseudomonas pseudomallei* it was found that high concentrations of ethanol precipitated the toxin. However, direct addition of ethanol to culture filtrates resulted in such dilute suspensions that the toxin was not readily recovered and conventional dialysis of the filtrates against ethanol, as described previously[1], was suitable only for the processing of small batches. An apparatus, was, therefore, developed which made it possible to precipitate the toxin from large volumes of filtrate by counter-current dialysis. Since the apparatus may be useful in numerous procedures where a high concentration of a dialysable precipitant is required, its construction and operation is described.

As shown in Fig. 1, the apparatus consists essentially of glass cylinders within which the dialysis tubing is suspended. The figure shows only 2 dialysis chambers but any number may be joined in series. The two cylinders (2 ft. long) made from 45-mm glass tubing were found to be suitable for use with either 27/32 or 36/32 in. Visking dialysis tubing. Gum-rubber tubing was used to connect the various sections of 8-mm tubing. The flow rates of ethanol and filtrate were independently regulated by an electrolytically controlled device[2] but other control methods could be employed. A slight difference in pressure between the inside and outside of the dialysis tubing was maintained by using the discharge tube of the effluent dialysate as a barometric leg so that the dialysis bags were kept distended. By arranging the flow of liquids as indicated in Fig. 1, the efficiency of counter-current operation was further increased by taking advantage of the convective flow caused by changes in density of each solution during dialysis.



Results of a typical dialysis experiment using 8 ft. of dialysis tubing in 6 cylinders are shown in Table I. In this experiment the cylinders were filled with ethanol, and the filtrate was introduced at a rapid rate until about one-half of the dialysis bags were filled; then the rate was reduced to about 40 ml/h. About 8 h later the flow of ethanol was started at about 25 ml/h. After about 24 h the composition of the effluent solutions became essentially constant. Density measurements of the effluent solutions

with a hydrometer served as convenient guides for adjusting the flow rate of either filtrate or ethanol to obtain the desired concentration of ethanol in the toxin suspension. Although it was generally unnecessary, the operation of the system was monitored by determining the density of the effluent filtrate every few days. After the flow rates were adjusted the only attention required was that of refilling the reservoirs and collecting the precipitate.

TABLE I

PRECIPITATION OF TOXIN BY CONTINUOUS COUNTER-CURRENT DIALYSIS

A series of 6 bags made of 27/32-in. tubing, each about 16 in long, was used. The system was operated for 7 days in a 2° water bath with a crude filtrate of *Pseudomonas pseudomallei* being added at about 40 ml/h and 95 % ethanol added at about 25 ml/h.

Solution	*Volume ml*	*Approx.* ethanol concentration %*
Influent filtrate	7,100	0
Influent ethanol	4,800	95
Effluent toxin suspension	2,100	85
Effluent dialysate	9,600	28

* Estimated from specific-gravity measurements.

An indication of the efficiency of the apparatus is obtained by comparing the low ethanol concentration in the waste dialysate with the high ethanol concentration in the concentrated toxin suspension (Table I). The concentration of ethanol in the effluent toxin suspension was not a direct function of the flow rate of the filtrate because changing the flow rate from 40 to 80 ml/h changed the ethanol concentration of the effluent toxin suspension from 85 to 70 %. The amount of filtrate that could be processed per hour was approx. proportional to the length of dialysis tubing.

Since the exact final concentration of ethanol in the effluent solutions cannot easily be predicted for any set of operating conditions, it is obvious that the continuous dialysis apparatus is not suitable for precise fractionation procedures. However, the apparatus is extremely useful for precipitating substances which are precipitable only at very high alcohol concentrations. The precipitation of toxin by dialysis against ethanol was desirable because the reduction in volume as well as in density of the solution facilitated the removal of the precipitate by centrifugation.

The advantages of using the apparatus for continuous counter-current dialysis over direct addition of alcohol or batch dialysis are: (a) solutions can be raised to high alcohol concentrations readily; (b) continuous flow facilitates the handling of large volumes; (c) a minimal volume of alcohol is required.

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