# CONTINUOUS COUNTERCURRENT DIALYSIS OF LARGE VOLUMES

by

Stanley Mandeles and Ernest C. Woods,
Space Sciences Laboratory
University of California
Berkeley, California

# CONTINUOUS COUNTERCURRENT DIALYSIS

The determination of base sequences in nucleic acids often involves the chromatographic separation of large quantities of oligonucleotides. The removal of salt from these chromatographic fractions by dialysis poses a problem when the volumes to be dialyzed exceed several hundred ml. We have constructed a dialyzer from plastic materials that removes solutes more rapidly than the usual multi-step dialysis method (1) and compares favorably in efficiency with the recently described thin film countercurrent dialyzer of Craig and Stewart (2).

## MATERIALS AND METHODS

In general, the dialyzer consists of an inner dialysis tube(through which the retentate flows) concentric with an outer vinyl tube (through which the diffusate flows). The inner dialysis tube is held in place by plastic spacers at two foot intervals to minimize kinking. This assembly can be extended for 20 or 30 feet, and is terminated at each end with a plastic connector with tubulature that permits separate inflow and outflow of retentate and diffusate. See Figure 1.

The following material is required for the construction of a single dialyzer unit 20 feet in length; 20 feet of tygon or vinyl plastic tubing, 1/2 " I.D., 5/8" O.D.; 2 feet of vinyl tubing, 1/8" I.D., 1/4" O.D.; 25 feet of 6 mm dialysis tubing; 12 inches of polyethylene tubing, 1/2" I.D., 5/8" O.D.; 4 inches of polyethylene rod, 7/8" O.D., polyethylene sheet, 1/8" x 4" x 4".

Construction of the Dialyzer. The details of construction are illustrated in Figures 1 - 4. Tygon or vinyl tubing, 1/2" I.D. is cut into two-foot lengths. The polyethylene tubing, 5/8" O.D., is cut into one-inch lengths and shallow grooves are cut in the plastic near the ends. Nine discs are cut from the 1/8" thick plastic sheet with 0.51" O.D. and a 1/4" hole in the center, and six or eight small perforating holes are drilled around the center as shown in Figure 2. Each disc is then pressed midway into a plastic connector so that the plane of the disc is perpendicular to the axis of the plastic connector tube. This is illustrated in Figure 2.

The end pieces are machined from the 7/8" plastic stock according to the dimensions shown in Figure 3. The retentate inlet and outlet tubes are made of the 1/4" O.D. vinyl plastic tubing. The diffusate inlet and outlet tubing may also be made of 1/4" vinyl tubing or machined as shown in Figure 3.

Assembly. The assembled dialyzer together with some of the details of the connections are shown in Figure 4. The assembly procedure involves threading the dialysis tubing alternately through the 1/2" vinyl tubing and the polyethylene connectors. It has been found convenient to fill the 20 foot length of dialysis tubing with water and then tie off the ends. This semi-rigid tube is moistened with water on the outside and can be threaded through the vinyl tubing and polyethylene connectors more easily. The ends of the vinyl tubing are then forced over the ends of the P.E. connectors. Care should be taken during this procedure to see that the dialysis tubing is not twisted. When all of the dialysis tubing has been threaded through the ten sections of vinyl tubing and nine polyethylene connectors and the appropriate connections made, the end pieces are prepared for connection by forcing a length of 1/4" O.D. vinyl tubing through the hole in the end piece provided for retentate flow. The 1/4" vinyl plastic tubing should extend about an inch beyond each end of the end piece. The dialysis tubing from the main assembly is then cut so that it extends about one inch beyond the edge of the 1/2" vinyl tube. The cut end of the dialysis tube is then forced over the end of the 1/4" vinyl tube from the end piece. The dialysis tube is secured in place with an "O" ring previously mounted on the 1/4" vinyl tube for this purpose or by several turns of Teflon covered 18 gauge copper wire. The 1/2" vinyl tubing is then forced over the end piece while taking care to draw the 1/4" vinyl retentate tubing out part way through the retentate hole in order to prevent twists and kinks. The same procedure is followed for the opposite end. In the instances where the diffusate outlet hole in the end piece has not been provided with a machined polyethylene tube, a simple outlet tube may be made by forcing 1/4" O.D. vinyl tubing to a depth of between 1/4" and 3/8" into the outlet hole made slightly smaller than 1/4".

Because the dialyzer is flexible, it may be arranged in any convenient configuration provided that there are no kinks or twists that will occlude flow. One satisfactory arrangement is that of a helix 12" in diameter and a 3" pitch. The helix permits the diffusate stream to be led in through the bottom and out through the top and this has been found helpful in 1) removing air bubbles when the source of the diffusate is the distilled water line or the de-ionized tap water line, and 2) mounting additional units for increased capacity. The retentate stream can be gravity fed or pumped in through the top and out through the bottom. When a pump is used, the dialysis can be made continuous by connecting the outlet of the retentate to the inlet of the pump. A reservoir may be provided for retentate volumes that are larger than the capacity of the unit being used (about 200 ml for the 20 foot unit). The progress of the dialysis can be continuously monitored, usually at or near the reservoir, for changes such as decreases in conductivity, or in visible or ultraviolet light absorption.

Dialysis of Solutions. The dialyzer was tested by observing the rate of dialysis of solutions commonly encountered in nucleic acid sequence determinations: KCl 1M; urea, 7M; adenosine, 0.1 mg/ml; oligo-adenylic acid of chain lengths $A_7$ to $A_{11}$, 0.1 mg/ml of 0.01 M $NaHCO_3$. In each case, 220 ml of solution was dialyzed in a single unit with a capacity of 200 ml. The retentate outlet was connected to a reservoir which was in series with a variable speed peristaltic pump. The pump outlet was connected to the retentate inlet so that the retentate could be cycled continuously through the dialyzer. The dialysis was carried out at 23$^{o}$C and at designated intervals, 1 - 3 ml of retentate was removed at the reservoir for analysis. The concentrations of each solute was determined as follows: KCl by conductivity, urea by weighing, adenosine and oligo-adenylic acid by spectrophotometry at 260 mμ. The flow rate of the retentate was approximately 10 ml/min, while that of the diffusate was 30 - 40 ml/min.

## RESULTS

The results of all four experiments are shown in Figure 5. In this graph, the log of the percent concentration of solute in the retentate is plotted versus time in hours. The concentration of solute at 0 time is set equal to 100%. It can be seen that the rate of dialysis is essentially a straight line in this plot, in accordance with theory. Oligo-adenylic acid is virtually not dialysable in eight hours, while 99% of the adenosine escaped in 7 hours. The rates for 1M KCl and 7M urea were greater: 99% of these solutes escaped in 2 1/3 hours (140 minutes).

## DISCUSSION

The continuous countercurrent dialyzer described above is a simply fabricated, easily assembled device. The only instance where the facilities of a machine shop is necessary is in the fashioning of the end pieces. We have found, however, that suitable end pieces can be fabricated of plastic T-tubes, 1/2" O.D., and various sized vinyl tubes telescoped to provide the same function as the machined end pieces. The device is portable, can be used in a number of different configurations, and is expandible for various volumes of retentate. We have found, however, that increasing the volume of the retentate without augmenting the dialysis capacity by additional units results in a proportional increase in the time of dialysis. The dialyzer is stable and may be used many times over. On the other hand, the dialysis tubing used with the apparatus can be chemically treated in order to alter the selectivity of the membrane (3).

In regard to the efficiency of the apparatus, a comparison may be made with the efficiency of the dialyzer, described by Craig and Stewart (2), that can clear over 99% of a 1M NaCl solution in a single pass through the device. The flow rates reported for this dialysis rate were between 0.5 and 1.0 ml/min. This means that, in order to clear 99% of solute from 200 ml of 1M NaCl, between 200 and 400 minutes of dialysis time is required. The dialyzer described here clears this amount in 140 minutes, providing an advantage for larger volume solutions. The disadvantages are that five to ten times as much diffusate is required to accomplish the same efficiency as the dialyzer of Craig and Stewart. In addition, the membrane area (1230 $cm^2$) is about three-fold greater than the thin film dialyzer membrane area, so that the opportunity to denature very fragile solutes is less in the thin film dialyzer. It is possible that smaller volumes may be handled and a higher rate of dialysis achieved with dialysis tubing of diameter smaller than 6 mm.

## SUMMARY

A simply fabricated, easily constructed continuous countercurrent dialyzer is described. The performance of this dialyzer was tested with various solutes. It was found to be very efficient for the dialysis of comparatively large volumes

## ACKNOWLEDGEMENTS

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3. Craig, L.C., and King, T.P., Methods of Biochemical Analysis, 10, 175, (1962).

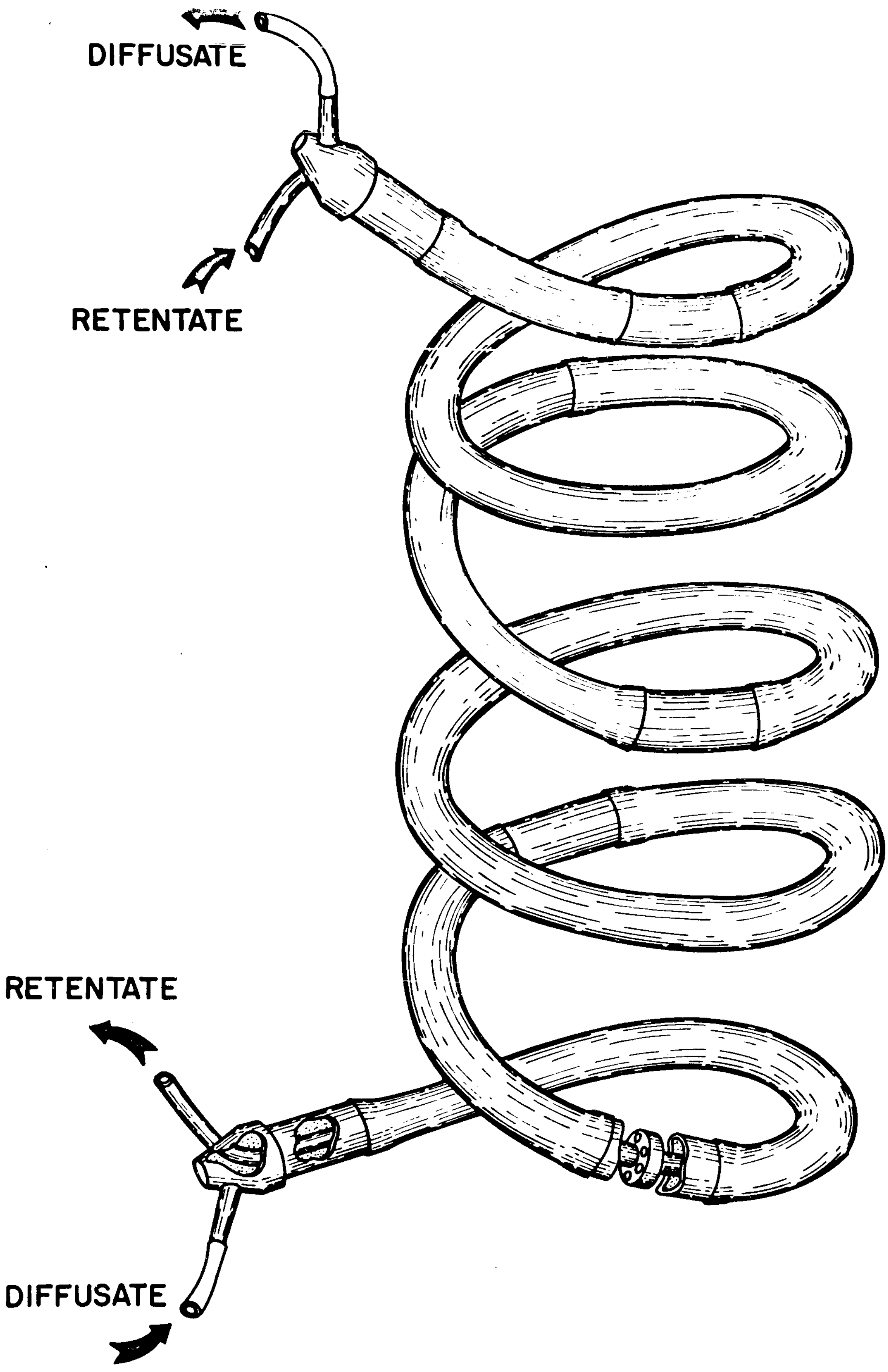
Figure 1. Assembled dialyzer. Cut-away of one connector and one end piece shown. Outer tube made of vinyl tubing, inner tube is 6 mm dialysis tube.

Figure 2. Dimensions of perforated disc and plastic connector. Cross-section of connector shown with disc in place.

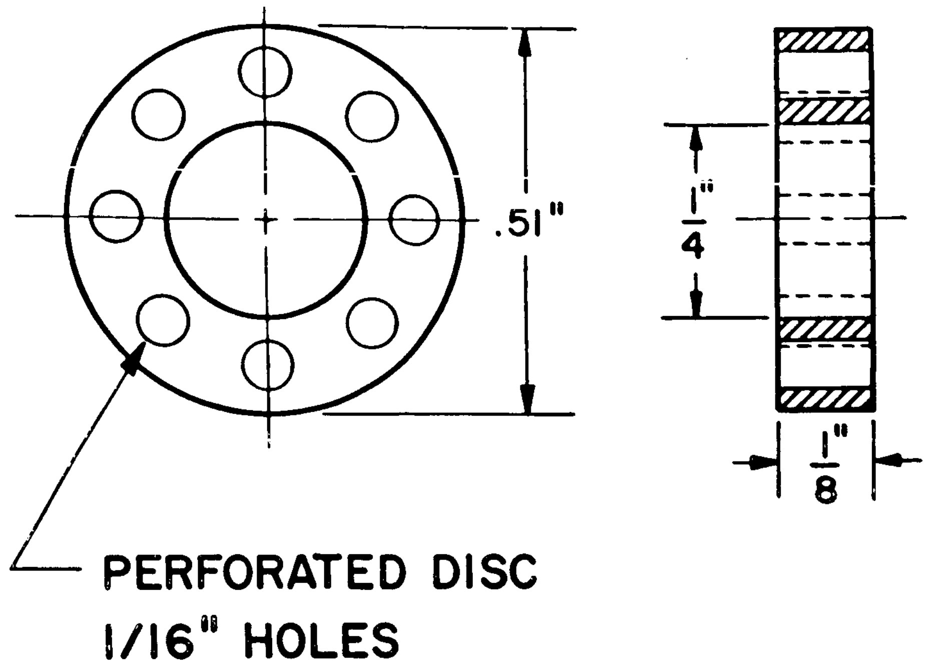
Figure 3. Dimensions of polyethylene end piece shown in cross-section of side view, and end view.

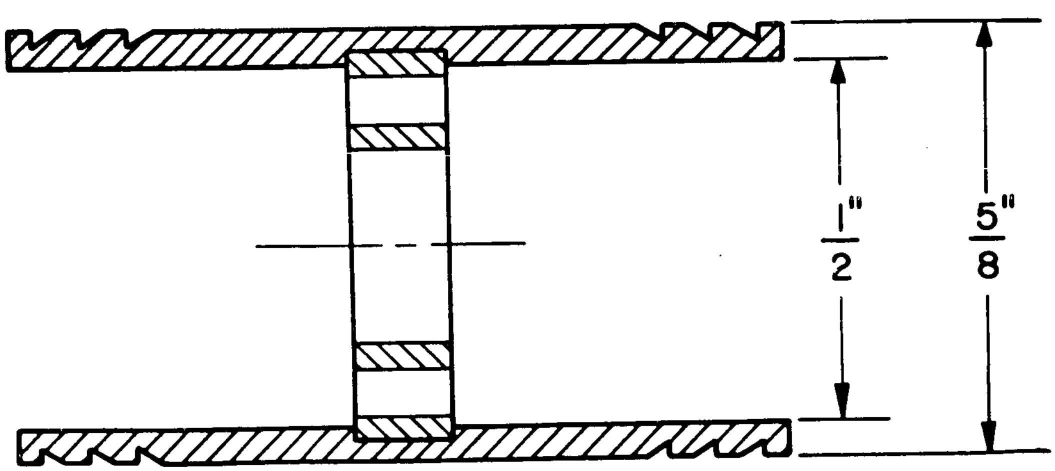
Figure 4. Cut-away and cross-section of connections in final assembly: (A) 1/4" O.D. polyethylene tubing; (B) end piece; (C) 1/4" O.D. vinyl tubing; (D) O-ring or Teflon-covered 18 gauge copper wire; (E) connector ; (F) 6 mm dialysis tubing; (G) 1/2" I.D. vinyl tubing.

Figure 5. Rate of dialysis of various solutes. Ordinate: percent of original solute concentration in retentate. Abscissa: time of dialysis in hours. Dialysis carried out at 23°C.

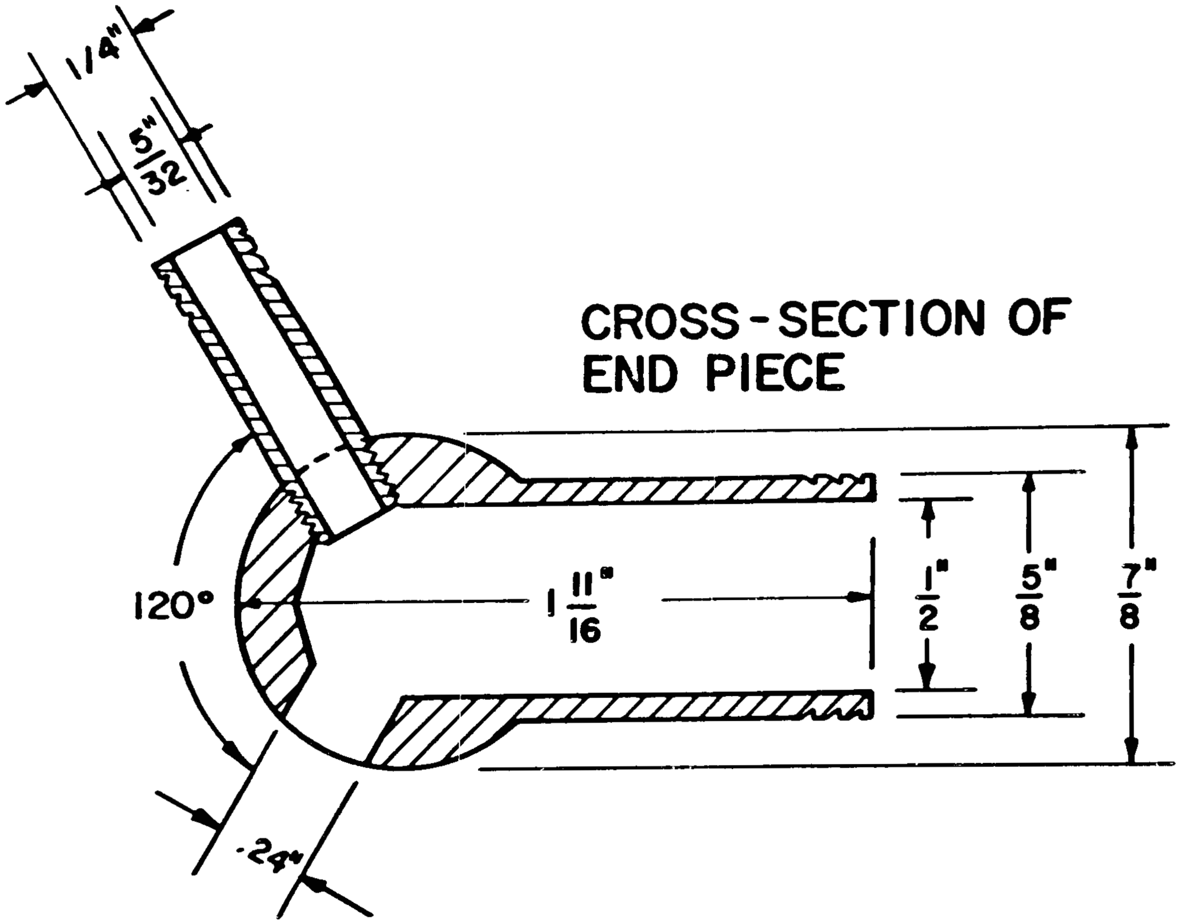



CONTINUOUS COUNTER-CURRENT DIALYZER

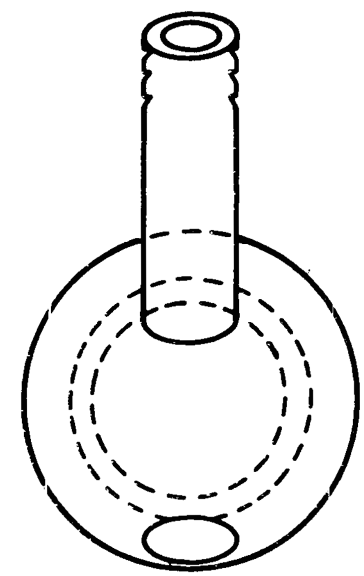


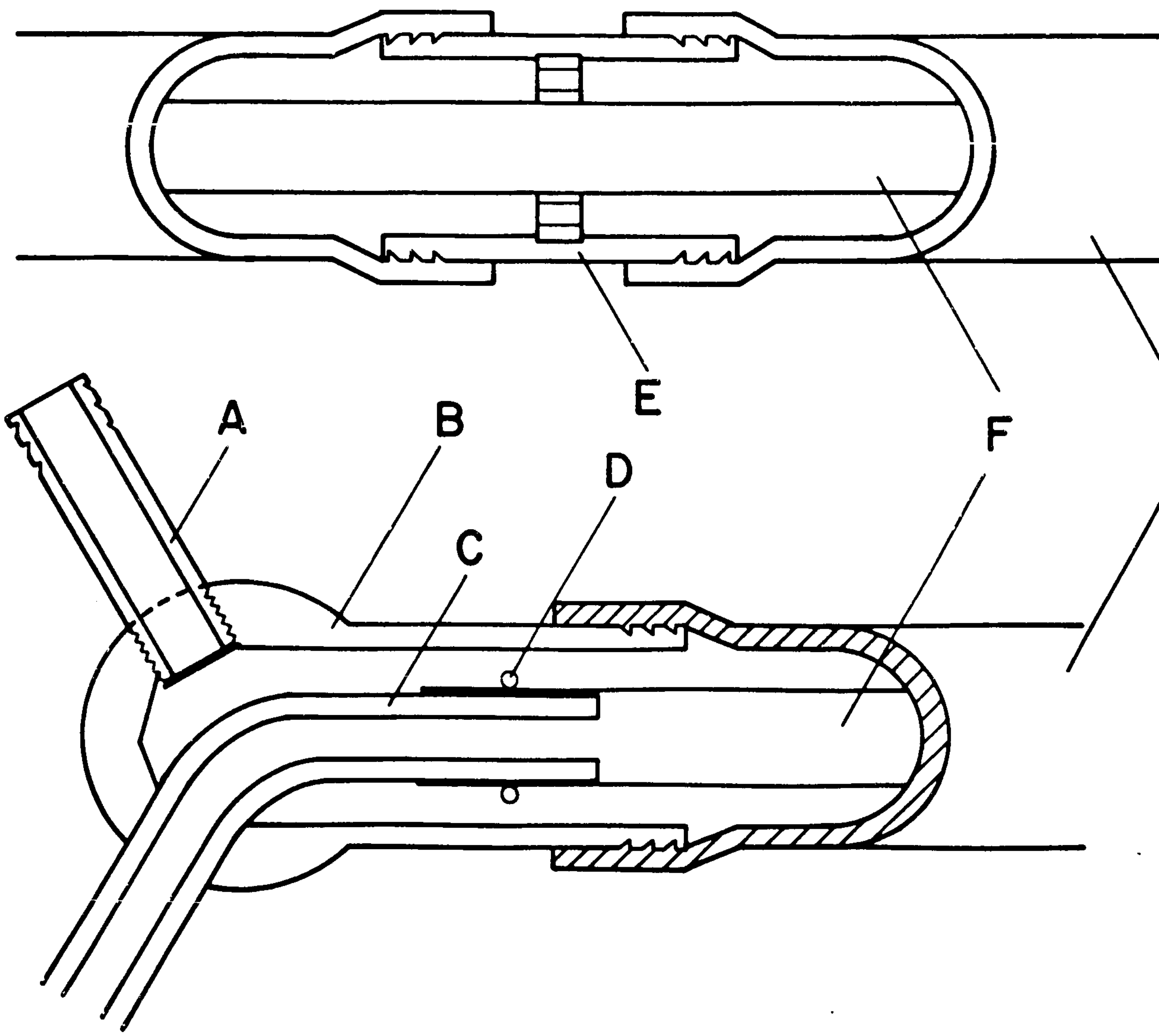

CROSS SECTION OF CONNECTOR
WITH DISC IN PLACE




CROSS-SECTION OF END PIECE



END VIEW


E
A
B
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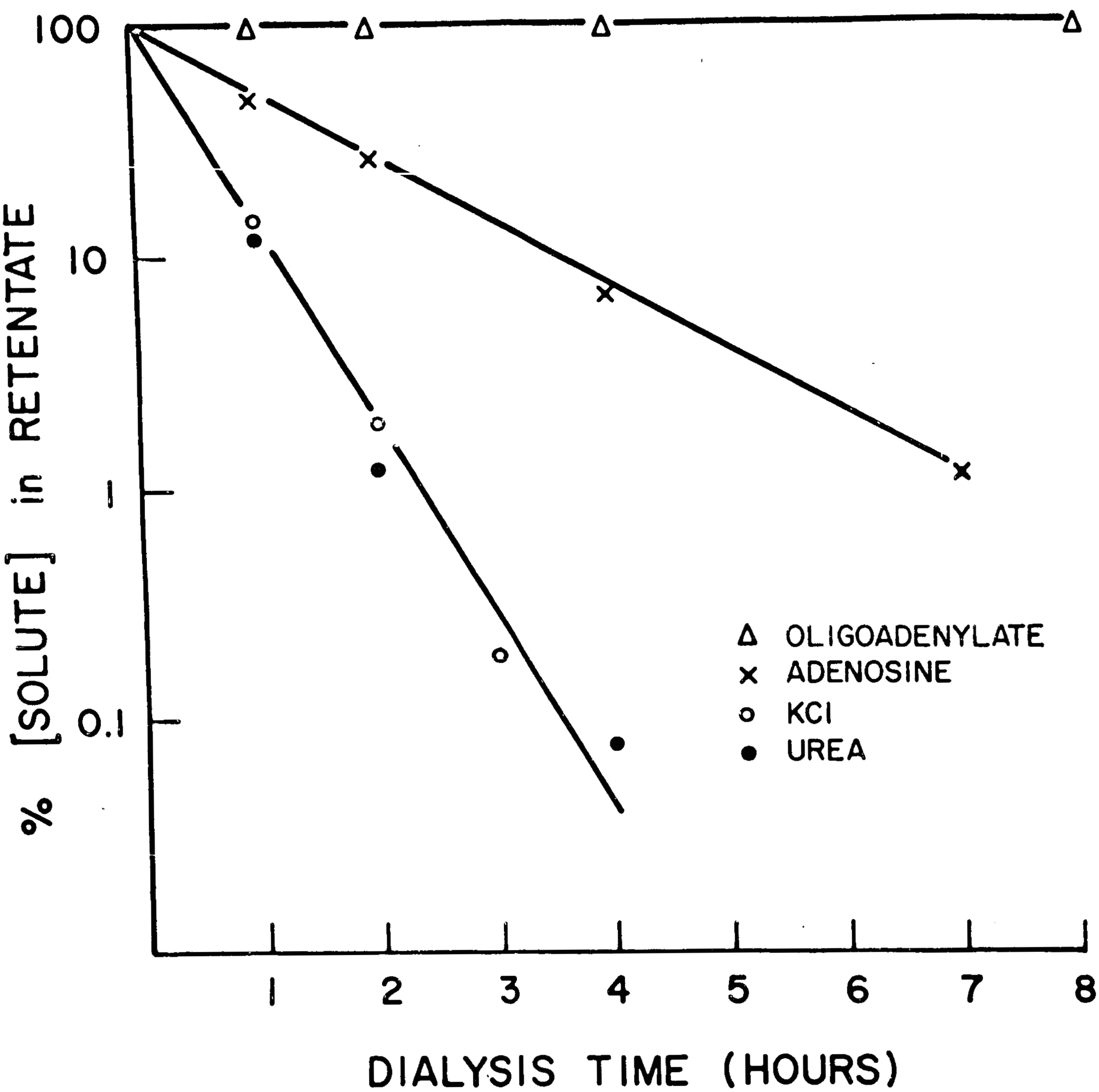
100
10
1
0.1
% [SOLUTE] in RETENTATE
1
2
3
4
5
6
7
8
DIALYSIS TIME (HOURS)
Δ OLIGOADENYLATE
× ADENOSINE
○ KCl
● UREA