Dialysis. X. On Thin Film Countercurrent Dialysis*

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ABSTRACT: A practical device for dialysis has been designed and built; it utilizes the advantages of thin film dialysis in a continuous feed, countercurrent system. Test data are given for a variety of solutes of different molecular weight.

The effects of temperature, concentration, rate of flow of solutions, and membrane porosity have been studied. The performance data suggest that the practical value of dialysis in biochemistry can be considerably improved.

From the standpoint of biochemistry and physiology there are many cogent reasons for studying dialysis and filtration in a continuously flowing system even though several fundamental and frustrating difficulties have not yet been entirely overcome in a practical laboratory apparatus. Theoretically there is the intriguing and selective way living tissues accept or reject from the blood stream precisely the solutes they require. Interest from this standpoint should not be entirely suppressed by the accepted view that so-called "active transport" is known to be involved in providing the selectivity and maintaining concentration gradients. Irrespective of this perhaps debatable point there is currently much interest (Working Conference on Hemodialysis, National Institutes of Health, Nov. 9–10, 1964) in developing an artificial kidney of small size which is based on the simple dialysis of a stream of blood diverted from the body through the dialyzer and returned again.

Perhaps most important as far as biochemistry is concerned is the prospect that a practical continuous dialysis column will be a very useful tool for rapidly removing the salt from the various concentrated salt solutions that are becoming ever more widely used and important in the fractionation of peptides, proteins, and nucleic acids. Scarcely less important for certain purposes is the prospect of selectively removing certain salts or components from solution without disturbing the concentration of others. Finally, the prospect of concentrating solutions of labile substances by dialysis against a solution of a polymeric substance of high osmolarity should be explored further.

Theoretically in an exchange process involving continuous flow the countercurrent arrangement should always be more effective than the cocurrent one, but for practical reasons it has not always been the one of choice. Thus most of the so-called artificial kidneys have employed a relatively large volume of solution on the "diffusate" side with no provision for its continuous removal. Saroff and Dillard (1952) published an ingenious design which provided a large dialyzing surface but operated by cocurrent flow with a very large ratio of the diffusate solution to the retentate.

Signer *et al.* (1946) made extensive studies with a stage continuous type of countercurrent train in which a concentration step for the diffusate solution followed each dialysis unit. Unfortunately the over-all operation was slow and the required data could not be obtained in a short time. More recently Vink (1962) has described a smaller stage continuous countercurrent apparatus also with a concentration step following each dialysis unit. His apparatus has been tested in the fractionation of polymers but not with the solutes biochemists are likely to use. Extensive mathematical treatment of the process was given by von Tavel (1947), Vink (1962), and Kastenbaum (1960).

The aim in designing the countercurrent apparatus used in the present study was to provide an exchange rapid enough so that the flow (and the total volume) of the diffusate stream would not have to be more than two- or threefold that of the retentate stream. On the basis of our experience with thin "film dialysis" for analytical purposes it seemed that this could be accomplished best by approaching as nearly as possible to the thin film concept. Partial success along this line has been achieved. The present model is a simple design that can be tested conveniently and rather rapidly with various solutes at any temperature desired.

Materials and Methods

The tryptophan, sodium chloride, ammonium sulfate, and sucrose were purchased from several different supply houses. The bacitracin was obtained from Commercial Solvents Corp., Terre Haute, Ind. (Lot B-55-10), and the subtilin was obtained from the Western Regional Laboratory of the U. S. Department of Agriculture. The cellophane tubing was of the $^{18}/_{32}$ and $^{20}/_{32}$ size and was purchased from the Visking Co., Chicago, Ill., during the years 1959 and 1964. The normal urine was obtained from a healthy male with no history of

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kidney malfunction. The pathological urine was from a patient with multiple myeloma.

The column for thin film continuous feed countercurrent dialysis is shown in Figure 1. The solution is pumped into the top of the capillary tube of the inside spacer tube, travels through the capillary tube, and comes into contact with the dialysis membrane at the bottom of the inside spacer tube. The solution then travels up the inside of the dialysis membrane in a thin film and exits at the retentate exit. The solvent is fed through a syphon tube to the top of the outside tube and travels down the outside of the dialysis membrane and through the diffusate exit at the bottom of the outside tube. The diffusate solution is then passed through the diffusate pump and into the diffusate collection flask. The outside tube rotates to maintain the uniform thin film on both the diffusate and the retentate sides of the dialysis membrane.

The apparatus consists of the following parts: (a) the inside spacer tube 106 cm long and 15 mm od with a Becton-Dickson standard ground glass male adapter joint at the top and sealed to a 1-mm capillary tube running the length of the inside of the tube; (b) a glass collar 8 cm long with retentate exit tube and with the top 3 cm having an id of 24 mm (not shown in the drawing) and the rest of the collar with an id of 18 mm (the membrane is attached to this collar. The membrane area is approximately 435 cm^2); (c) the rotating outside tube of length 97 cm, of which the top 5 cm has an id of 24 mm and the remainder of the tube has an id of 15.6 mm. (A Becton-Dickson male ground glass adapter is sealed to the bottom of the outside tube. The bearing at the bottom of the outside tube consists of a No. 19 Luer stainless steel needle about 3 cm in length inserted into the capillary Teflon tubing. This connection can be clamped with the weight of the apparatus on the needle in the tubing which provides the seal for this bearing. The distances between the inside spacer tube and the outside tube are critical. A clearance of 0.5 ± 0.1 mm between the inside spacer tube and the outside tube has been found to be satisfactory; (d) a bearing and support for the outside tube which consists of a small Teflon-covered wire ring; (e) the drive belt; "O" rings (C. E. Conover and Co., Caldwell, N. J., part 2-244, compound N109-7) have been used, although they have a short lifetime; rubber bands are completely unsatisfactory; (f) a constant speed motor to give 150 rpm of the outside tube; the Bodine motor type CRGL has been found to be satisfactory; (g) the retentate and diffusate pump; one pump was used to pump both the retentate and the diffusate solutions; the Micro Bilateral Roller Pump, of the Holter Co. (Bridgeport, Pa.), Model RD044, was found to be satisfactory when used with the Silastic pumping tubes PT090 (retentate solution) and PT250 (diffusate solution).

When dialyses were run at temperatures other than ambient, the dialyzer was placed in a water jacket (not shown in the drawing) with the bottom bearing of the dialyzer passing through a rubber stopper. The top of the water jacket was open. A variable speed pump (Mannostat by E. Greinier and Company, New York,

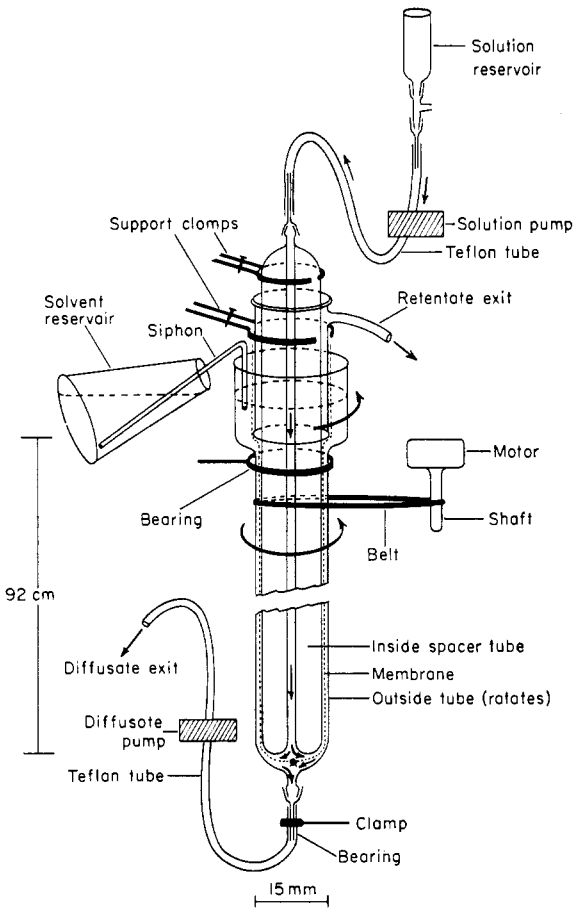

FIGURE 1: Schematic drawing of thin film dialyzer.

N. Y.) was used for water circulation. The water jacket was 95 cm long and had an id of 31 mm.

The outside tube is placed in a vertical position through the upper Teflon-covered bearing which holds the outside tube in the correct upright position. A No. 19 Luer stainless steel needle with the point cut off is placed on the Becton-Dickson adapter at the bottom of the outside tube and inserted into the capillary Teflon tubing which leads to the pump. The tubing with the needle surrounded by the rubber stopper of the outer water jacket provides the support at the bottom of the tube. When the assembly is finished the outside tube should be freely rotating on the vertical axis. The Teflon-steel bearing gives little friction, particularly when the tube is partially supported by the buoyancy of the water in the temperature control jacket.

The appropriate dialysis tubing is wet with tap water and stretched moderately by hand. The tubing should be stretched until it just fits over the inside spacer tube. If the dialysis membrane can be rotated around the spacer tube with the appearance of ridges, the membrane is too loose. The membrane is then gently pushed over the bottom edge of the collar and pushed up the collar until it comes to the bottom of the delivery tube of the collar. The inside spacer tube is then inserted

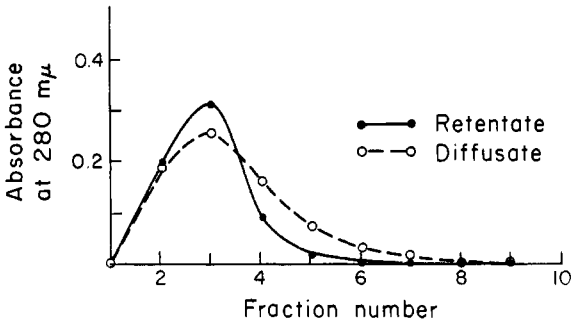

FIGURE 2: Plot showing performance of dialyzer with a single injection of tryptophan as solute. Retentate/diffusate flow rates = 2.5/2.5.

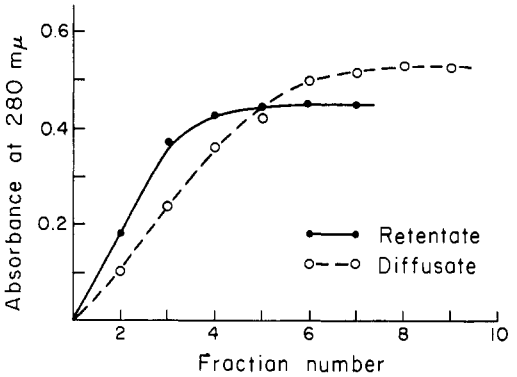

FIGURE 3: Plot showing performance of dialyzer with continuous injection of tryptophan as solute. Retentate/diffusate flow rates = approximately $^1/_2$.

through the collar into the membrane until about 1 in. of the membrane is not filled by the spacer tube. The end of the membrane is then tied with No. 00 surgeon's silk thread in two consecutive knots. The unused membrane is cut off and the remainder of the membrane is slipped over the spacer tube. The membrane must be snug with little or no play. The assembly which includes the spacer tube, the collar, and the membrane is then inserted into the outside tube. The collar is clamped and held so that its bottom is about 1 cm above the narrow portion of the outside tube and is centered. The inside spacer tube is gently and firmly pushed through the collar, thus stretching the membrane, until the slack is removed and the membrane is under slight tension. The inside spacer tube is then clamped in position, care being taken that it is centered inside the collar.

The bottom of the dialysis membrane and inside spacer tube should be quite near the bottom of the outside tube to minimize the dead volume. Some space must be left so that the membrane does not touch the bottom of the outside tube. A No. 18 Luer stainless steel needle is attached to the Becton-Dickson adapter at the top of the inside spacer tube, and the appropriate tubing is attached to the needle.

A flexible polyethylene siphon tube is inserted into the space between the collar and the outside tube and held in place with a rubber band. The bottom of the siphon tube should not extend below the collar. A drive belt is placed below the ring bearing with the other end around the shaft of a constant speed motor.

In evaluating the dialysis column the following solutions were dialyzed against deionized water: 1 M sodium chloride, 50% saturated ammonium sulfate, 3% saturated ammonium sulfate, 2.46×10^{-3} M tryptophan, and 1.92×10^{-3} M bacitracin. The subtilin solution, 2.42×10^{-3} M in 0.01 N acetic acid, was dialyzed against 0.01 N acetic acid.

The following effects were studied: variation in the temperature, variation in the flow rate at constant ratio of diffusate to retentate flow rates, and variation in the flow rate ratio.

The concentrations of the tryptophan, bacitracin, and subtilin solutions were determined spectrophotometrically, the concentrations of the sodium chloride and ammonium sulfate solutions were determined conductometrically. Optical rotation was used for the measurement of the sucrose concentrations. The salt concentrations of the urine samples were estimated

TABLE 1: Dialysis Data Obtained with the Thin Film Countercurrent Dialyzer at 3° and the Intermediate Pump Setting.

Solutes	Size 18			Size 20		
	Exit Flow Rate		Removal (%)	Exit Flow Rate		Removal (%)
	Retentate	Diffusate		Retentate	Diffusate	
Tryptophan (2.46×10^{-3} M)	0.46	1.22	84.7	0.40	1.46	84.3
Sucrose (6%)	0.51	1.39	67.0			
Bacitracin (1.92×10^{-3} M)	0.47	1.22	46.5	0.42	1.40	52.8
Subtilin (2.42×10^{-3} M)	0.45	1.22	19.0	0.43	1.35	31.5
1 M NaCl	0.53	1.23	97.7	0.40	1.38	99.0
3% satd. $(NH_4)_2SO_4$	0.51	1.23	92.8	0.36	0.90	97.9
50% satd. $(NH_4)_2SO_4$	0.81	1.15	89.6			

TABLE II: Dialysis Data Obtained with the Thin Film Countercurrent Dialyzer at 25° and the Faster Pump Setting.

Solutes	Size 18			Size 20		
	Exit Flow Rate		Removal (%)	Exit Flow Rate		Removal (%)
	Retentate	Diffusate		Retentate	Diffusate	
Tryptophan (2.46×10^{-3} M)	0.61	1.59	87.2	0.65	1.80	87.0
Sucrose (6%)	0.69	1.85	79.0			
Sucrose (30%)	0.96	1.80	68.4			
Bacitracin (1.92×10^{-3} M)	0.61	1.59	54.0	0.65	1.96	64.0
1 M NaCl	0.73	1.68	98.0	0.80	1.92	98.8
3% satd. $(NH_4)_2SO_4$	0.66	1.62	94.9	0.58	1.90	98.7
50% satd. $(NH_4)_2SO_4$	1.15	1.59	88.0	1.05	1.75	91.0

TABLE III: Dialysis Data Obtained with the Thin Film Countercurrent Dialyzer at 25° and the Intermediate Pump Setting.

Solutes	Size 18			Size 20		
	Exit Flow Rate		Removal (%)	Exit Flow Rate		Removal (%)
	Retentate	Dif-fusate		Retentate	Dif-fusate	
Tryptophan (2.46×10^{-3} M)	0.45	1.19	91.6	0.45	1.32	93.9
Sucrose (6%)	0.52	1.42	82.6			
Sucrose (30%)	0.75	1.37	72.7			
Bacitracin (1.92×10^{-3} M)	0.46	1.20	60.7	0.48	1.48	67.4
Subtilin (2.42×10^{-3} M)	0.46	1.28	30.0	0.45	1.43	44.2
1 M NaCl	0.54	1.25	99.4	0.56	1.46	99.4
3% satd. $(NH_4)_2SO_4$	0.50	1.20	96.9	0.56	1.45	98.5
50% satd. $(NH_4)_2SO_4$	0.86	1.18	91.8	0.94	1.43	91.5

conductometrically, and also the weight was determined after lyophilization.

The first preliminary evaluation of the dialyzer was made by allowing 3–5 ml of a known concentration of tryptophan to enter as the retentate stream and by measurement of the solute concentrations which emerged in both the retentate and diffusate streams. This evaluation was followed by the more significant one of measuring the steady-state retentate and diffusate concentration levels when solution and solvent were continuously fed into the column.

Results

In Figure 2 the results of the dialysis of a 3-ml injected solution of tryptophan are shown. The flow rates of the two streams were each about 2.5 ml/min with 5-ml fractions collected from each exit stream. The absorbance at 280 mμ of each successive fraction was determined and plotted. This typical plot indicates that solute interchange is rapid and that such a solute is cleared from the column by about 15–20 ml of solvent. The buildup of the solute in the diffusate requires a similar volume; as can be seen in Figure 2 both curves are almost symmetrical.

In Figure 3 typical results of the continuous feed dialysis are shown. As expected approximately 15–20 ml of solution in the retentate stream is required to reach the steady state.

Other experiments have indicated that from 5 to 7 ml of solution are in contact with the membrane at any given time. Depending on the flow rate any part of the solution is in contact with the membrane for about 20 min.

The results of the dialysis of several solutions are given in Tables I to V. The retentate and diffusate exit flow rates are given in ml/min. The values given for the per cent removal are those found when the steady state has been obtained. Values are given for the dialyses with two different Visking cellophanes, No. 18 and 20. These membranes have approximately the same diameter when wet, but they differ markedly in porosity, as shown in previous papers (Craig *et al.*, 1957; Craig and Konigsberg, 1961). The No. 18 membrane is the less porous of the two cellophanes.

Table I contains the results of the dialyses at 2–3°

TABLE IV: Dialysis Data Obtained with the Thin Film Countercurrent Dialyzer at 25° and the Slower Pump Setting.

Solutes	Size 18			Size 20		
	Exit Flow Rate		Removal (%)	Exit Flow Rate		Removal (%)
	Retentate	Diffusate		Retentate	Diffusate	
Tryptophan (2.46×10^{-3} M)	0.36	1.00	94.2			
Sucrose (6%)	0.43	1.16	86.1			
Bacitracin (1.92×10^{-3} M)	0.37	0.97	64.0	0.39	1.25	70.5
1 M NaCl	0.44	1.00	99.7	0.33	1.18	99.7
3% satd. $(NH_4)_2SO_4$	0.43	1.00	97.9	0.48	1.20	98.7
50% satd. $(NH_4)_2SO_4$	0.73	0.98	94.3	0.40	1.18	93.1

TABLE V: Dialysis Data Obtained with the Thin Film Countercurrent Dialyzer at 40° and the Faster Pump Setting.

Solutes	Size 18			Size 20		
	Exit Flow Rate		Removal (%)	Exit Flow Rate		Removal (%)
	Retentate	Diffusate		Retentate	Diffusate	
Tryptophan (2.46×10^{-3} M)	0.61	1.63	93.6	0.64	1.95	92.3
Sucrose (6%)	0.70	1.90	79.7			
Bacitracin (1.92×10^{-3} M)	0.59	1.55	67.5	0.65	2.00	67.8
Subtilin (2.42×10^{-3} M)	0.61	1.70	43.0	0.58	1.87	50.0
1 M NaCl	0.71	1.64	99.4	0.75	1.99	99.6
3% satd. $(NH_4)_2SO_4$	0.67	1.62	97.4			

and an intermediate pump setting. Tables II, III, and IV give similar results for the dialyses done at 25° at different pump settings. Table V contains the results of the dialyses at 40° and the highest pump setting used. The results given in these tables were obtained from dialyses on one apparatus and with the same membrane for the dialyses of each of the solutions under the varying conditions of temperature and flow rate.

The results of the dialyses of both normal and pathological urine are given in Table VI. In each case the urine was passed through the dialyzer at 25° at a retentate flow of 0.69 ml/min and a diffusate flow rate of 1.85 ml/min.

TABLE VI: Results of the Dialyses of Urine with the Countercurrent Thin Film Dialyzer.

Sample	Removal (%) (conductance)	Removal (%) (wt)	Retentate Solids (g/100 ml of starting material)
Normal urine			
No treatment	0	0	2.831
1st Pass	92.7	96.1	.111
2nd Pass	98.0	98.7	.038
Myeloma urine			
No treatment	0	0	2.856
1st Pass	95.4	85.3	.419
2nd Pass	98.9	90.8	.263

Discussion

In numerous previous papers (Craig, 1964) it has been shown that high selectivity in differential dialysis requires that the free diffusion of the solutes across the membrane be restricted to a considerable degree. This requires that the process be slow per unit area of dialyzing surface, but the over-all process need not be slow provided sufficient dialyzing surface per unit volume of the solution is employed, as is the case when a thin film of the solution is spread over a relatively large membrane surface. Such an arrangement bypasses the difficulty of the quiescent solution film next the membrane (Ginzburg and Katchalsky (1963)), which has concerned physiologists and chemical engineers for so long.

This requirement imposes experimental difficulties easily overcome on an analytical scale (Craig and Konigsberg, (1961)), but much more difficult to overcome in the design of a continuous countercurrent

process for preparative purposes. There is the problem of maintaining thin solution films of reproducible thickness flowing counter to each other with only a flexible cellophane membrane separating them. The flow must be uniform, without channeling, and of a controllable steady rate.

It seemed that this might be accomplished between two glass tubes, one sufficiently smaller than the other so that with the smaller one properly spaced inside the larger an annular space of less than 1 mm in thickness would be provided. With dialysis casing over the smaller one two annular spaces separated by the membrane would then be provided whose thickness could vary only through the movement of the membrane. In this case the thickness of the one could only vary at the expense of the other. The arrangement in Figure 1 is based on this principle and shows the way of accomplishing the countercurrent flow. The rotating outer tube, among other things, is a self-centering device.

Even selected glass tubing is not sufficiently uniform to provide an even annular thickness of less than 1 mm, and therefore channeling would be expected. In the beginning it was hoped that this difficulty could be overcome by causing the two concentric glass tubes to rotate in opposite directions at an adjustable relative speed so that the membrane would be held stationary between them with support required only at the top of the sac.

Actually such an apparatus with a dialyzing surface about 20 cm in length was constructed and found to be effective. However, when the length of the dialyzing surface was increased it was found too difficult to keep the membrane from twisting, thereby defeating the purpose.

An alternative scheme was then tried. The diameter of the wet casing was adjusted slightly by longitudinal stretching while wet so that it would fit over the inner tube with a slight tension. Then when solution was forced between it and the membrane through the capillary central tube the desired uniform film would be produced for the inner film. Channeling in the outer film and, apparently due to the flexibility of the membrane, in the inner as well, is prevented by rotating only the outer tube. This scheme is the one used in Figure 1. When operation was attempted without rotating the outer tube the efficiency fell markedly. Moreover, comparison of the apparatus of Figure 1 which has a 92-cm length of dialysis sac with that of an earlier 20-cm model in which rotation in opposite directions was employed indicated that the efficiency of the longer tube was roughly proportional to the length.

While a still longer tube could be built this would not be as easy to build and control. It would seem more profitable to investigate a number of such units operating in series in a countercurrent manner. This promising possibility will be studied in the future.

The mathematical interpretation of countercurrent dialysis has been treated in part by von Tavel (1947), Vink (1962), and Kastenbaum (1960). Just as the interpretation of continuous extraction column processes are based on analogy to the stepwise process which corresponds to countercurrent distribution, so can continuous dialysis be interpreted by the stepwise process (Craig and King, 1955) based on the binomial expansion. Analogy to the counter double current distribution apparatus (Post and Craig, 1963), in which the feed solution is one of the phases being introduced at the appropriate end of the train, is of interest here. No attempt will be made here to develop the analogy further. The rates of flow of the two films and the rate of diffusion across the membrane can be considered as determining the concentration gradients for a given small section of the column which might be thought of as analogous to a partition ratio in counter double current distribution. From this analogy it is easy to see that a higher rate of flow for the diffusate film is highly desirable. On the other hand, looking ahead to the use of several such columns in series and for other practical reasons including recovery of solute in the diffusate, it is desirable to minimize dilution. A compromise was reached in which a diffusate flow rate between two- and threefold that of the retentate has been selected for study in the present work.

Several consequences of the restricted thickness of the films and the flexibility of the membrane soon became apparent during the experimental tests. The pressure required to cause an aqueous solution of the retentate to flow through at a rate of 0.5–1.0 ml/min approximated 50 cm of water, and the diffuse exit tube had to be lowered a similar distance or a little more below the top level of the entering diffusate solution. Therefore the retentate film thickness must be greater at the bottom of the column than at the top, while that of the diffusate film must be the reverse. Obviously the viscosity of the solution is important here, and there is an optimum rate of flow of the two film streams above which the efficiency rapidly decreases. These limits were found to be just above the rates shown in Tables I–V. The data in Tables II–IV also, as expected, show a dependence of the efficiency on flow rate at a fixed ratio of flow rates.

It was rather surprising to find the degree of filtration from the retentate to the diffusate film. This was detected by measuring the inlet and outlet flow of the retentate stream. It amounted to 9–15% of the volume and therefore must be considered an appreciable factor in the operation of the column.

On the other hand, when the solution to be dialyzed had a high salt content while the diffusate solution had little, the retentate stream increased in volume, as reflected in the flow rate, due to osmotic flow against the rather low pressure. This is shown in the data with 50% saturated ammonium sulfate in Tables I–IV and explains in part why the per cent removal of the half-saturated ammonium sulfate solution is lower than that of 3% saturated solution.

It was rather surprising that a change of temperature did not have more influence on the performance of the dialyzer. The rate of diffusion according to the Stokes–Einstein relationship changes in proportion to the absolute temperature and inversely as the viscosity. This should favor dialysis for an ideal solute by a factor

of 1.96 for the temperature interval 3–25° and by 1.44 for the interval 25–40°. In analytical thin film dialysis where the porosity is such that the solute can barely pass, coefficients larger than these are usually observed. It is therefore surprising that a change in temperature has so little effect on the rate of dialysis in the continuous dialyzer. Nonetheless a higher temperature does speed up the process and favors removal of a solute from the retentate. While the solutes tested all diffuse readily through the membrane, it is interesting that the higher temperature offers an advantage less than that expected from the Stokes–Einstein relationship. This may be of a certain theoretical interest and could be interpreted as indicating that this type of restricted diffusion is more efficient at the lower temperature than expected. Such behavior was noted irrespective of the molecular size of the solute. Lyman *et al.* (1964) in dialysis studies with a porous membrane also noted low temperature coefficients.

The purpose of the present study is not to develop an apparatus best suited for fractionation of closely related solutes but rather for rapid removal of salts, urea, and other low molecular weight components of a solvent system. Nonetheless it does have a certain promise in connection with fractionation where the relatively low dilution on each pass is optimal for countercurrent operation. The over-all selectivity of a single pass through the dialyzer as indicated by the comparison of bacitracin and subtilin in the tables is somewhat less than that shown by the analytical thin film dialysis, but the process can be preparative and the separation should be markedly improved by the proper use of several units operating countercurrently. This possibility, the use of membranes better suited from the standpoint of porosity and the use of salts and urea as solution and conformation modifiers, will be the subject of a future study.

It seemed of interest to determine how efficient the dialyzer would be for removing urea and salts from urine. Accordingly a sample of normal fresh urine was passed through the dialyzer at 25° at a retentate flow rate of 0.69 ml/min while the diffusate flow rate was 1.85 ml/min. The salt removal was estimated by conductance and the total solids by weight after lyophilization.

For comparison a sample of pathological urine from a patient with multiple myeloma (Type 1) was also studied in the same way. The data are given in Table VI. As expected, the pathological urine had much more nondialyzable material, probably mostly Bence-Jones protein. Of considerable interest is the contrast of conductance and residue weight which in the case of the second pass with the pathological urine can indicate considerable peptide material of molecular weight range 1000–3000 and a way of obtaining it rather easily.

It is of some interest to compare the efficiency of the present dialysis column with those described previously. Direct comparisons, however, are not easy since most of them have not been operated countercurrently. The relative flow rates and the test solutes are all different. A direct comparison can be made, however, with the column proposed by Saroff and Dillard (1952) since they studied solutions of sodium chloride and sucrose. However, cocurrent operation was used and the column had a much larger membrane surface. At a flow rate of 2 ml/min for the retentate and a much higher rate for the diffusate a 1:1 mixture of molar solutions of salt and sucrose gave a retentate in which 14% of sugar was retained with a molar ratio of sucrose to salt of 18:1. From the data in Table III it can be estimated that the present dialyzer would give a retentate with 27% of the sucrose at a molar ratio of sucrose to salt of 45:1 in a single pass and with the flow rate of the diffusate stream only 2.3 times that of the retentate.

There are many attractive features connected with a small dialyzer of the type described in this paper. It is a relatively rapid operation. Therefore solutes of poor stability can be dialyzed at a considerably higher temperature where they need be exposed to the column temperature only 10–20 min. This can be important for removing solutes more tightly bound at the lower temperature. It is effective for concentrated salt solutions. Danger of loss of valuable components is minimized since the dilution is minimal. A considerably wider range of molecular sizes can be studied without fear of undue loss.

Finally it seems reasonable to suggest that the principle of the thin film and countercurrent flow might be adapted to the design of an "artificial kidney." The theoretical requirements and experimental difficulties to be overcome have been well treated by Leonard and Bluemle (1959) and by Wolf *et al.* (1951). For comparison Wolf *et al.* report a removal of about 60% of the sodium chloride from a sample of "blood" in one pass through a conventional artificial kidney. The dialyzer in Figure 1 will remove over 99% sodium chloride from a 1 N aqueous solution in a single pass.

Another basis for comparison might be taken from the recent data of Maxwell and Moffitt (1965) obtained with the Technicon dialyzer. One pass removed approximately 20% of the amino acids from milk and 12 passes are required to remove 93–95%. From Table III it will be seen that this percentage of removal from water is obtained with tryptophan on a single pass in the dialyzer described here.

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Chlorogenic Acid Biosynthesis. Chemical Synthesis and Properties of the Mono-*O*-cinnamoylquinic Acids*

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ABSTRACT: Rational syntheses of the 1- and 3-*O*-cinnamoylquinic acids are reported. Negligible wandering of the cinnamoyl group occurred under the mildly acidic conditions used to remove the protecting groups from the substituted quinic acid molecule. The 4- and 5-*O*-cinnamoylquinic acids were isolated from the mixture formed when barium hydroxide acted on 1-*O*-cinnamoylquinide. Once the lactone ring was opened, the cinnamoyl group migrated from hydroxyl to hydroxyl group.

The results of experiments with [U-^{14}C]quinic acid establish that the base-catalyzed migration of cinnamoyl groups about the quinic acid molecule is an intramolecular process. Three lines of evidence establish that migrations occur in the sequence 1 $\rightleftharpoons$ 5 $\rightleftharpoons$ 4 $\rightleftharpoons$ 3: (a) studies of migration when the carboxyl is methylated; (b) studies of migration when the 4,5-hydroxyl groups are blocked by an isopropylidene group; and (c) studies of the rates of migration in buffered solutions (pH 6–10). It is improbable that a 1 $\rightarrow$ 3 migration, by way of a cinnamic–quinic mixed anhydride, is of importance.

Chlorogenic acid (3-*O*-caffeoylquinic acid), and a variety of closely related hydroxycinnamoyl conjugates, are widely distributed in the roots, stems, leaves, and flowers of plants (Herrmann, 1956; Sondheimer, 1964). Despite the ubiquitous occurrence of these compounds their functions in the life of the plant and the main facts concerning their biosynthesis have yet to be established. The compounds, or their derivatives, may play an important role in morphogenesis; thus they may act as mediators of photoperiodic effects (Zucker *et al.*, 1965; Bottomley *et al.*, 1965) and be associated with the action of plant growth hormones (Zenk and Müller, 1963). They may also act as cofactors in oxidation–reduction systems (Marrè *et al.*, 1962) or in photophosphorylation (Haberman, 1963), and they may influence the activity of specific enzymes or types of enzyme (Schwimmer, 1958).

Levy and Zucker (1960) postulated that 3-*O*-cinnamoyl- and 3-*O*-*p*-coumaroylquinic acids are intermediates in the biosynthesis of chlorogenic acid. In order to investigate this hypothesis further it was necessary to prepare 3-*O*-cinnamoylquinic acid by rational chemical synthesis. At an early point in the investigation it became apparent that the cinnamoyl group is liable to migrate from hydroxyl to hydroxyl group about the quinic acid molecule. To prove conclusively that the 3-isomer had been synthesized all four mono-*O*-cinnamoylquinic acids had to be obtained and characterized. The present paper describes (1) the synthesis of these isomers, and (2) a study of the base-catalyzed process by which cinnamoyl group migration occurs.

Related problems in the synthesis of the *p*-coumaroyl, caffeoyl, and galloyl esters of quinic acid have been concurrently explored in two laboratories. Investigators at the University of Rome have reported the synthesis of 1,4-di- (Panizzi *et al.*, 1954); 1-, and 4,5-di- (Scarpati *et al.*, 1958); 3- (Panizzi *et al.*, 1956); and 4-, 5-, and 1,3-di-*O*-caffeoylquinic acids (Scarpati *et al.* 1964). In view of later work by this group (Scarpati and Esposito, 1964) and results reported in the present paper, mixtures of isomers were probably obtained in the synthesis of the 1,4-di- and 3-*O*-caffeoylquinic acids. The Sheffield University group has synthesized 3-*O*-*p*-coumaroylquinic acid (Haslam *et al.*, 1961); 1-, 4-, and 5-*O*-*p*-coumaroylquinic acids; and 5-*O*-caffeoylquinic

* From the Biochemistry Department, The Connecticut Agricultural Experiment Station, New Haven, Conn. *Received June 25, 1965.* This work was supported, in part, by grant GB-2626 from the National Science Foundation. A preliminary account of the synthetic studies has appeared (Hanson, 1963). The evidence for assigning an absolute configuration to quinic acid has been reviewed (Hanson, 1962). The various carbon atoms in the cyclohexane ring of quinic acid are indicated by the system of stereospecific numbering generally employed for this compound.