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The Dialysis of Non-Electrolytes Through Regenerated Cellulose (Cuprophan). I. The Effect of Molecular Size

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Synopsis

Solutions of single solutes were dialyzed through Cuprophan using a modified Muir-Ross cell and their half-clearance times were measured. A linear relationship was found between half-clearance time and molecular cross-sectional area for solutes with molecular weights between 60 and 1134. The size of the largest molecule which will dialyze through Cuprophan probably has a molecular weight of about 18,000.

INTRODUCTION

It is known that a large number of compounds are removed from the plasma of uremic subjects by hemodialysis.[1] Many of these have molecular weights less than 200, and the fate of such solutes as urea, creatinine, sodium, potassium, chloride, and amino acids has been well documented.[2,3] However, little is known about the removal of larger molecules. Although compounds with molecular weights as high as 130,000 will dialyze through chemically treated cellophane membranes,[4] there is no clear indication of the size of the largest solute which will dialyze through untreated cellophane. The present work was undertaken to clarify this problem by dialyzing solutions of compounds with molecular weights up to 17,800 through Cuprophan, the membrane commonly used for hemodialysis. A modified version of the Muir-Ross cell was used for this purpose. An attempt was made to find a relationship between dialysis rate and molecular size.

EXPERIMENTAL

The design of the side chambers of the Muir-Ross dialysis cell[5] was simplified and the cell lid was altered to allow the inclusion of a temperature probe and two tubes through which the solution could be recirculated from the cell body.[6] The modified cell is shown in Figure 1. Distilled, deionized water (termed the dialysis fluid), 6.5 liters, was maintained at 37.5°C

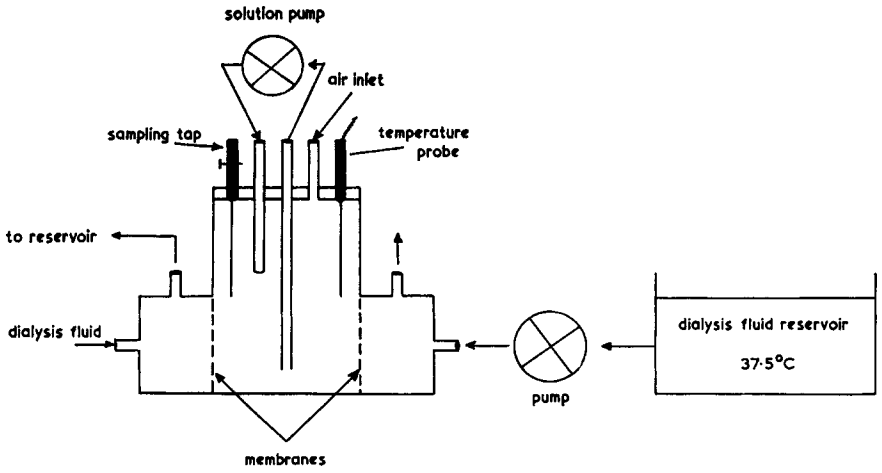

Fig. 1. Experimental arrangement for dialysis in the modified Muir-Ross cell. Procedure for dialysis was as outlined in text.

in a reservoir and recirculated through the side chambers of the dialysis cell. The test solution (usually containing 1 g/l. of solute), 230 ml, in distilled, deionized water and heated to 37.5°C, was placed in the body of the cell which was then pressurized to 100 mm Hg to keep the membrane flat against its support. The cell was placed in a water bath at 37.5°C, and samples of solution were withdrawn at the beginning and at regular intervals during the experiment. Both the test solution and dialysis fluid were recirculated at 300 ml/min.

The simplest factor for expressing dialysis rate in the Muir-Ross cell is the "half-clearance time" ($t_{1/2}$), defined as the time taken for the concentration of the solute to fall to half its initial value. Since the solute concentration decreases exponentially with time, $t_{1/2}$ can be found from a graph of log solute concentration against time and is given by

$$t_{1/2} = 0.301/k$$

where k is the slope of the graph. In all experiments, a correction was applied to the measured solute concentration to allow for the loss in volume of the solution due to sampling and ultrafiltration under the applied pressure. The 95% confidence limits of $t_{1/2}$ were estimated from the 95% confidence limits (i.e., mean ± 2 S.D.) of the analytical methods used to determine the concentrations of the solutes.

The membrane, Cuprophan (Bemberg A.G., Wuppertal, West Germany), was a regenerated cellulose membrane of dry thickness 10.5–11.5 μm and is normally used with the Kiil and Rone-Poulenc hemodialyzers.

Inulin was recrystallized from water,[7] but other solutes were used in the purest commercially available form. Urea, creatinine, and glucose were determined by standard Technicon AutoAnalyzer procedures (Technicon Instruments Co. Ltd., Basingstoke, Hants). Maltose, maltotriose,

maltotetraose, maltopentaose, maltohexaose, and cycloheptaamylose were determined as glucose after hydrolysis with 1*N* sulfuric acid. Fructose, sucrose, raffinose, and inulin were determined as fructose by the HCl/alcoholic resorcinol reaction of Kulka.[8] Myoglobin concentrations were measured from absorbance readings at 410 nm.

RESULTS

A series of experiments were carried out to test the effect of minor variations in the experimental conditions. Using solutions of urea, creatinine, and raffinose, it was shown that $t_{1/2}$ was independent of both the initial concentration (range 0.25–12.00 g/l.) of the solute and the pressure (range 50–200 mm Hg) applied to the solution. The values of half-clearance time were found to be reproducible to within the estimated 95% confidence limits. For example, the mean $t_{1/2}$ for urea in four experiments was found to be 76.2 min (range 74.3–78.2 min). The confidence limits of a single estimate were ±5 min. It was further shown that the half-clearance times of urea, creatinine, sucrose, and raffinose decreased with temperature, and the relationship conformed to the Stokes-Einstein diffusion equation[9] over the range 27.5°–47.5°C.

The influence of molecular size on $t_{1/2}$ was studied by dialyzing solutions of urea, creatinine, glucose, fructose, maltose, sucrose, maltotriose, raffinose, maltotetraose, maltopentaose, maltohexaose, cycloheptaamylose, inulin,

TABLE I
Half-Clearance Times and Molecular-Size Parameters of Nonelectrolytes Dialyzed Through Cuprophan[a]

Solute	$t_{1/2}$, min	M_r	πr^2, nm^2	$r^{3/2}$, nm$^{3/2}$	log $t_{1/2}$	log M_r
Urea	76.2 ± 5 (4)	60	2.09	0.414	1.882	1.778
Creatinine	111.4 ± 7 (4)	113	2.85	0.527	2.047	2.053
Glucose	170.5 ± 10 (2)	180	4.00	0.674	2.232	2.255
Fructose	189.6 ± 14 (3)	180	3.96	0.669	2.278	2.255
Maltose	281.3 ± 13 (2)	342	6.05	0.920	2.449	2.534
Sucrose	311.5 ± 22 (2)	342	6.33	0.951	2.493	2.534
Maltotriose	441 ± 25 (2)	504	9.26	1.265	2.644	2.702
Raffinose	440.8 ± 30 (2)	594	9.95	1.336	2.644	2.774
Maltotetraose	510 ± 30 (2)	666	11.68	1.507	2.708	2.823
Maltopentaose	614 ± 36 (2)	828	13.68	1.696	2.788	2.918
Maltohexaose	656 ± 37 (2)	990	16.69	1.968	2.817	2.996
Cycloheptaamylose	800 ± 36 (2)	1134	18.33	2.112	2.903	3.055
Inulin	7030 ± 480 (3)	5600	74.95	6.073	3.847	3.748
Myoglobin	3138 hr ± 399 hr (2)	17800	112.16	8.217	5.275	4.250

[a] Experimental conditions were as described in the text. Figures in parentheses denote the number of determinations. The 95% confidence limits of $t_{1/2}$, shown as ± values, were estimated as outlined in the text. The reference for the methods of calculation of πr^2 and $r^{3/2}$ is given in the text.

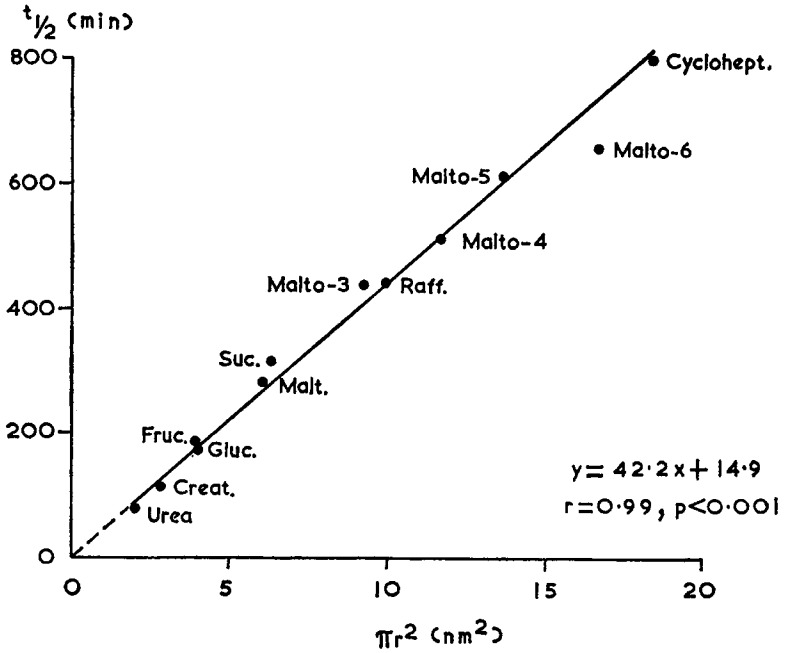

Fig. 2. Relationship between $t_{1/2}$ and molecular cross-sectional area. Experimental conditions were as described in text.

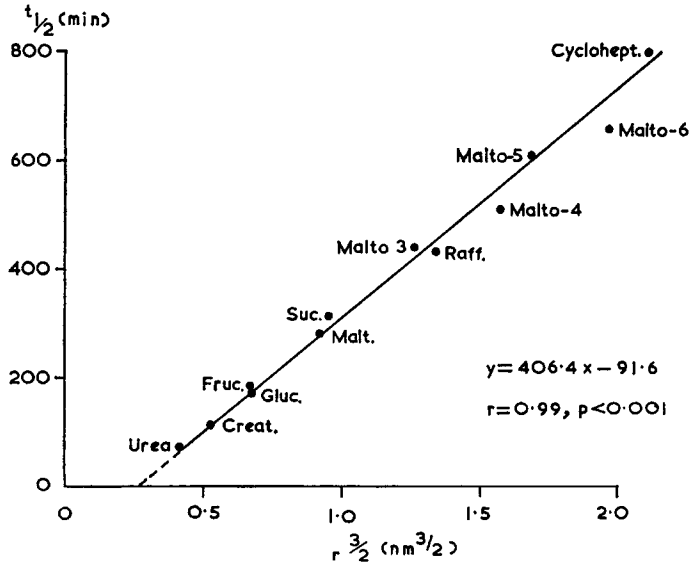

Fig. 3. Relationship between $t_{1/2}$ and square root of molecular volume (represented by $r^{3/2}$). Experimental conditions were as described in text.

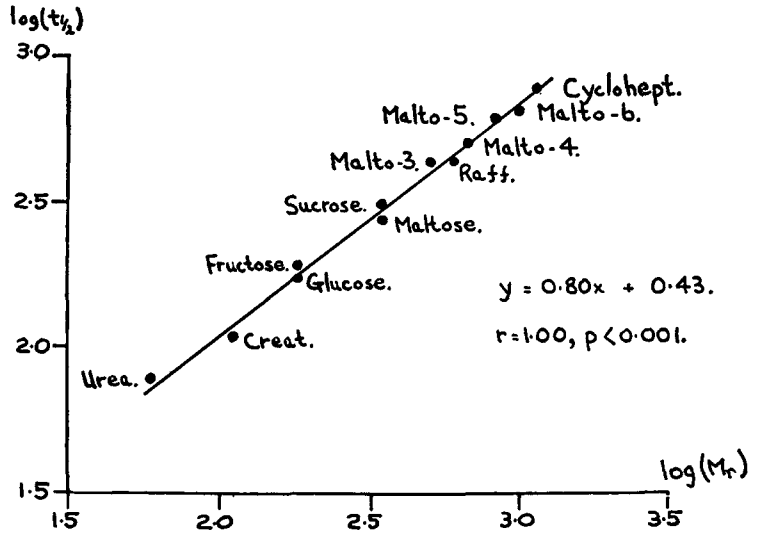

Fig. 4. Relationship between log $t_{1/2}$ and log molecular weight. Experimental conditions were as described in text.

and myoglobin. Treating the solutes as incompressible spheres, molecular radii (r) were derived from equations based on known physical properties of the solutes,[6] and various molecular-size parameters were then calculated. The parameters used were molecular cross-sectional area (πr^2)[6]; square root of molecular volume[10] (taken as $r^{3/2}$), which are compared with $t_{1/2}$ for the 14 solutes in Table I; and log molecular weight, which is compared with log $t_{1/2}$ in Table I. It was found that myoglobin adsorbed onto the membrane, and so its half-clearance time was estimated from the amount found in the dialysis fluid after 24 hr. The relationships between $t_{1/2}$ and πr^2 and $r^{3/2}$ and between log ($t_{1/2}$) and log molecular weight are shown in Figures 2, 3, and 4. There was a significantly linear correlation, passing through the origin, between $t_{1/2}$ and πr^2 for solutes with molecular weights between 60 and 1134. The square root of molecular volume showed a linear correlation with $t_{1/2}$ which did not pass through the origin (Fig. 3), and a significantly linear correlation was also found between log ($t_{1/2}$) and log molecular weight (Fig. 4). Correlations between $t_{1/2}$ and other molecular-size parameters, such as molecular volume ($^4/_3\pi r^3$)[11] and square root of molecular weight,[12] were found to be nonlinear.

The linear correlation between $t_{1/2}$ and πr^2 found for solutes between urea and cycloheptaamylose did not apply for inulin or myoglobin. When the calculated values of πr^2 for inulin and myoglobin were substituted in the regression equation given in Figure 2 the values of $t_{1/2}$ for these two compounds were 3178 and 4748 min, respectively. Similarly, when the values of log molecular weight for these two solutes were substituted in the regression equation given in Figure 4, their $t_{1/2}$ values were 2812 and 7129 min,

respectively. The values found by experiment were 7030 min and 3138 hr (188,280 min). Thus, values of $t_{1/2}$ for inulin and myoglobin are considerably higher than those predicted from their molecular size.

DISCUSSION

The dialysis rate of a solute depends both on its rate of entry into the membrane pores and its diffusion through them. Considering the solute as an incompressible sphere of radius r and the membrane pore as a cylinder (radius R), a mathematical function has been proposed relating the area of pore available for dialysis to the size of the solute[13]:

$$\frac{\text{Available pore area}}{\text{total pore area}} = \underbrace{(1 - r/R)^2}_{(1)} \underbrace{(1 - 2.104\,(r/R) + 2.95\,(r/R)^3 - 0.95\,(r/R)^5}_{(2)}$$

Term (1) of this multipower function takes into account the rate of entry of the solute into the pore, while term (2) is a measure of the friction occurring between the dialyzing solute and the pore walls. It has been shown that the friction in a membrane pore between a diffusing solute and the solvent molecules does not increase proportionately with solute size.[14] Thus, the relationship between molecular size and dialysis rate is complex and cannot be expressed as a simple, linear correlation between $t_{1/2}$ and a single molecular-size parameter. The good linear relationships found between $t_{1/2}$ and πr^2 and $r^{3/2}$ and between log $t_{1/2}$ and log molecular weight would appear to be fortuitous, and are obviously simplifications of the kinetics of dialysis. However, these empirical correlations do have some value in predicting the dialysis rates of nonelectrolytes with molecular weights up to at least 1134. The correlations may hold above this value, but many solutes with greater molecular weight are only stable in buffer solutions, and factors other than molecular size, e.g., the constituents of the buffer, may influence the dialysis rates of these solutes.

The values of πr^2 for inulin and myoglobin agree with molecular sizes calculated by other means.[7,15,16] Thus, the finding that these solutes show disproportionately high increases in $t_{1/2}$ with πr^2 is probably due to reasons other than molecular size. Although a pore size for Cuprophan can be estimated,[6] this is only an average value, and there is probably a broad range of pore sizes, as has been shown for other cellophane membranes.[13] Since solutes of molecular sizes between those of urea and cycloheptaamylose are appreciably smaller than the average pore size of Cuprophan (3.1 nm), it is unlikely that the heteroporosity of the membrane will influence to a significant degree the difference between the number of pores that urea and cycloheptaamylose may enter. However, inulin and myoglobin are solutes of large molecular size with sizes approaching that of the Cuprophan pore (r = 1.54 nm for inulin and 1.89 nm for myoglobin)[6]; and so it is likely that, because of the membrane heteroporosity, the number of pores which those two solutes can enter is less than for the other solutes. Thus, there may not

be a sharp cutoff in the size of the largest molecule which will dialyze through a membrane.

As the size of the solute approaches that of the membrane pores, the conformation of the solute in solution becomes a critical factor in determining whether it enters the pore. The importance of solute conformation has been demonstrated by Craig,[17] who added various salts to the solvent in which proteins dialyzed through cellophane, and by Osterhoudt[18] in dialyzing isomeric alcohols through cellulose acetate membranes. Therefore, solute conformation in solution will determine the dialysis rates of inulin and myoglobin to a greater extent than for the other solutes. The adsorption of myoglobin onto Cuprophan that was observed also accounts in part for the very slow dialysis rate of this solute. The size of the largest solute which will dialyze through Cuprophan is probably near that of myoglobin with a molecular weight of 18,000. This figure is in agreement with that of Alexander and Galletti,[19] who showed that ^{14}C-dextran (molecular weight 16,000–19,000) dialyzed across Cuprophan mounted in a Klung artificial kidney.

The findings presented in this paper indicate that solutes with molecular weights between 1134 and 18,000 may dialyze across Cuprophan under hemodialysis conditions. The nature and size of these compounds may be determined in a similar way to that carried out for compounds of low molecular weight[1] using techniques such as gel filtration, gas–liquid chromatography, and mass spectrometry.

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