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Differential Dialysis

Molecular size and structure can be correlated with diffusion rates through calibrated dialysis membranes.

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The question "Is it dialyzable?" is very often asked in connection with complex natural products of unknown structure. It is a question which usually can be answered very easily by an experimental test in the laboratory for those solutes which are of large molecular size and are totally excluded by the dialysis membrane, or for solutes of small molecular size which are readily diffusible. But for compounds with intermediate-sized molecules the test is not so decisive. Even solutes of relatively small molecular size sometimes mysteriously fail to dialyze. Nonetheless, dialysis is such a simple laboratory operation that it has seemed well worth while to try to make it a more discriminating tool. In fact, the discoverer of dialysis, Thomas Graham, was certain, about 100 years ago, that this would be very much worth while. However, as might be expected, the realization of such an objective is not simple, because the dialyzability of any solute is a function of the membrane and its properties, of the solute and its properties, of the particular solvent used, and of the way these three factors interact with each other.

It is at once obvious that we must deal ultimately with ambiguous, complex phenomena which have intrigued the best minds in physical chemistry and physiology for a very long time. Theories of diffusion, the complex nature of membranes, and theories of solutions are involved, and there is an enormous literature to be considered. It is not the purpose of this short article even to begin to review the literature (*1–4*) but only to present a few simple observations which have resulted from a purely experimental approach to the study of selectivity in dialysis.

There are several cogent reasons for attempting a study of this type. In the first place, little practical advance in the field has been made for the last 30 years, aside from the development of better membranes. In the meantime many new techniques have been developed which should be of help in gaining a better understanding. In addition, the structures of many solutes of relatively large molecular size have been at least partially elucidated. Although we still are far from a complete understanding of the size and shape of their molecules or of the way these solutes behave in solution, much helpful information is now at hand. It seems not too early to try to use them as model solutes for the study of membrane diffusion.

Secondly, from the standpoint of the separation of solutes of unknown structure and the problem of proving whether or not a given preparation is pure, it is important to be able to sort mixtures of solutes clearly on the basis of well-defined parameters such as size, charge, partition, volatility, solubility, and adsorbability, and to know which parameter really is responsible for the separation. The majority of the most selective separation methods in use today depend on combinations of such parameters and, in addition, are complex countercurrent processes. Here the rate of interchange between phases can be a deciding factor. In spite of a considerable body of theoretical information and a highly developed technology, the real and critical basis for the selectivity is still largely a matter of opinion.

Dialysis is clearly a diffusional process which depends on a particular rate of transport of the solute of interest. In this respect it is uncomplicated. If rates of dialysis could be correlated clearly with the size of the solute molecule, they should be of considerable interest. There would seem to be no question about the acceptability of such a correlation for estimating the molecular size (by order of magnitude) of a given unknown solute, but when an attempt is made to make more penetrating comparisons of molecules differing only slightly from each other, the basis becomes very much more uncertain. The present article is directed toward a discussion of this problem.

The effectiveness of differential dialysis when there are large differences in molecular size is primarily based on a sieving effect. When differences are smaller, the controlling factors become much more nearly related to differential diffusion and it is to be expected that the overall shape of the molecule will become important, perhaps even more important than the relative volumes of the solutes. Differential solvation effects also would be expected to play a role, and so would any inherent tendency of the solutes to associate to polymers. There is a definite possibility that, if the selectivity or discriminatory power of differential dialysis can be sufficiently improved, the method can be made of value for gaining information about these factors. This could be very worth while, particularly because of the lack of simple experimental methods wholly satisfactory for estimating the shape or conformation in solution of large and more or less flexible molecules.

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High Selectivity

Without pursuing such hypothetical possibilities further, at this point it seems well to consider first the problem of high selectivity and how it is to be achieved. There is also the problem of relating such selectivity clearly to a parameter I call "diffusional size." That is to say, the role, in the selectivity, of such membrane effects as fixed charge and adsorption must be eliminated or at least minimized. The term *diffusional size* is used here to indicate that size and shape both play a role. When the effective shape of the molecule differs appreciably from that of a sphere, rotational diffusion should make such a solute molecule behave as if it were a larger molecule.

Dialysis and ultrafiltration have always been studied together, since in theory they are closely related procedures (*5*). It has in the past been somewhat easier to develop a theory for the selectivity of ultrafiltration. The basis for discrimination with a simple sieve is obvious when the particles being sieved are relatively large. But as the particle size becomes smaller, finally reaching molecular dimensions, other factors enter, and the simple sieving effect becomes overshadowed by a host of other effects most of which stem from molecular interactions. A serious difficulty in ultrafiltration derives from the fact that the pores are somewhat distorted by the pressure and become more or less plugged as the filtration proceeds, thus altering the effective pore size. Dialysis is free from this disadvantage because here only diffusional activity would tend to cause a particle to enter a pore. If the particle should be too large to pass through, the diffusional activity of the solvent in the reverse direction should be effective in dislodging it.

Prior to the introduction of the ultracentrifuge there was considerable interest in ultrafiltration as a method for determining the size of large molecules. However, the promise of greater reliability and precision with the ultracentrifuge caused a decline of interest in ultrafiltration. Nonetheless, several excellent reviews (*2, 3, 5*) and papers (*4, 6*) were published which are still basic to the understanding of dialysis.

Perhaps the most important concept for the development of selectivity in dialysis is that relating to pore size. Admittedly the "pores," if we assume pores to be present, are irregular and of various sizes. In spite of this, a spherical molecule considerably smaller than the cross-sectional area of the mouth of a round "pore" would have nearly the entire pore area available for diffusion. But as the size of the pore becomes smaller and reaches the point where it is scarcely larger than the molecule, it is the difference between the cross-sectional size of the pore and that of the molecule which is important. A simple relationship proposed by Ferry (*7*) many years ago is given by Eq. 1:

$$A = A_0(1-r/a)^2 \qquad (1)$$

Here A is the area available for diffusion, A_0 is the cross-sectional area of the pore, r is the radius of the sphere (the molecule), and a is the radius of the pore.

Equation 1 requires that, as the limiting size of the pore is approached, a small increase in the size of the diffusing molecule will be sufficient to prevent it from entering the pore. In other words, the selectivity of membrane diffusion should become very much higher when the membrane will barely allow the solute of interest to diffuse through it.

In order to put this simple conclusion to a test, the most common dialysis tubing, Visking cellophane, may be used. Size 20 tubing will allow ribonuclease (molecular weight, 13,600) to pass very slowly at room temperature when the solvent is 0.01*N* acetic acid but will almost completely hold back chymotrypsinogen (molecular weight, 24,500). However, very little resolution will be noted with this membrane

Table 1. Comparison of relative diffusion coefficients D in free diffusion with relative dialysis rates.*

Substance	Molecular weight	10^{-6} D	D ratios	$T/2$† (hr)	$T/2$† (ratios)
		Section 1			
Xylose	150	7.462		1.3 (*1*)	
			1.02		1.46
Arabinose	150	7.599		1.9 (*1*)	
			1.13		1.84
Glucose	180	6.728		3.5 (*1*)	
			1.01		1.37
Galactose	180	6.655		4.8 (*1*)	
			1.28		6.2
Sucrose	342	5.209		30.0 (*1*)	
		Section 2			
Sucrose	342	5.209		1.5 (*2*)	
			1.03		1.73
Lactose	342	5.076		2.6 (*2*)	
			1.01		1.04
Cellobiose	342	5.039		2.7 (*2*)	
			1.16		2.0
Raffinose	504	4.339		5.4 (*2*)	
			1.13		2.8
Stachyose	666	3.839		15.0 (*2*)	
		Section 3			
Stachyose	666	3.839		0.68(*5*)	
			1.19		1.64
Cycloheptaamylose	1152	3.224		1.1	
		Section 4			
Cyclohexaamylose	972	3.443		6.0 (*3*)	
			1.07		2.0
Cycloheptaamylose	1152	3.224		12.0 (*3*)	
			1.07		1.91
Cycloöctaamylose	1296	3.000		23.0 (*3*)	

* The four sections in the table represent data from four different membranes. The ratios refer to the particular value over the next corresponding one in the series, for example, xylose/arabinose, arabinose/glucose, and so forth. † T/Z, 50 percent escape time.

Table 2. Comparative dialysis rates of tryptic peptides from hemoglobin.

Peptide	Number of residues	$T/2$ (hr)* In 0.01*N* AcOH, *p*H 3.3	$T/2$ (hr)* In 0.15*N* NH_4Ac, *p*H 5.8
+ − +− Val Asp Pro Val AspNH_2 Phe Lys	7	1.8	4.9
+ +− Leu Leu Val Val Tyr Pro Try Thr GluNH_2 Arg	10	6.0	5.8
+− +− Glu Phe Thr Pro Pro Val GluNH_2 Ala Ala Tyr GluNH_2 Lys	12	3.8	7.8
+ − − +− Val AspNH_2 Val Asp Glu Val Gly Gly Glu Ala Leu Gly Arg	13	5.1	12.8
+ + − − − +− Val Gly Ala His Ala Gly Glu Tyr Gly Ala Glu Ala Leu Glu Arg	15	7.4	17.0

* $T/2$, 50-percent escape time.

for a mixture of bacitracin (molecular weight, 1420) and subtilin (molecular weight, 3200). Both diffuse rapidly through the membrane.

Therefore, a relatively slow dialysis per square centimeter of membrane surface is the most promising approach for achieving high selectivity. In order to overcome the time disadvantage, the membrane area can be greatly increased in proportion to the volume of solution to be dialyzed. This solution we shall call the "retentate." The solution receiving the diffusate must be kept as dilute as possible. Experimentally, with Visking 20 tubing it has been possible to spread 0.5 milliliter of solution uniformly over about 50 square centimeters of membrane by inserting a closed glass tube of appropriate size into a sac made from the dialysis tubing. The solution is thus spread evenly over the membrane to a depth of about 0.1 millimeter. The rate of diffusion can then be determined by periodic analysis of the diffusate and replacement of it with fresh solvent. The details of this technique have been published (*8*) and need not be given here.

When the concentration of the diffusate is kept sufficiently low in relation to that of the retentate, the escape rate for a pure single solute should follow first-order kinetics. Many examples of this have now been published (*9*). One of them (*10*) is a comparison of glucose and sucrose, shown in Fig. 1. This type of chart is of considerable value in determining whether or not a given preparation behaves ideally and is homogeneous with respect to molecular size (*11*). Moreover, where the solutes give straight-line escape plots, the 50-percent escape time can be used for comparisons related to the sizes of the solute molecules. Conversely, where the sizes of the solute molecules are known, the ratio of the 50-percent escape times, for two solutes such as those of Fig. 1, clearly defines the selectivity of the membrane. The stage is thus set for a meaningful study of differential dialysis which does not require such variable concepts as "mean pore size," "tortuosity factors," "permeation coefficients," and "osmotic flow" (*5*, *12*). While the straight line (Fig. 1) is the composite result of many factors, it in fact gives the pertinent information needed. Adsorption in, or on, the membrane can be detected easily by simple recovery estimation.

Perhaps it is advisable to say a word or two about the membrane before developing the viewpoint to be presented here. Cellophane thus far has proved the most interesting membrane of all those available for laboratory dialysis. It has been shown that cellophane carries practically no fixed charge and does not give trouble due to adsorption for most solutes when 0.01*N* acetic acid is the solvent. It has been shown rather conclusively (*8*) that cellophane behaves as if it had "pores" of more or less fixed size, but it should be remembered that wet cellophane is in fact a gel. Some degree of flexibility in the pores might be expected, and any mechanical strain must be strictly avoided if the porosity is to be held constant. This is emphasized by the finding that a stretching procedure (*8*) can serve as an effective method for adjusting pore size. The matrix is probably something like a tangled mass of chains made from glucose units linked 1 to 4. In any case cellophane imbibes a considerable amount of water to form the spongelike medium characteristic of so many membranes.

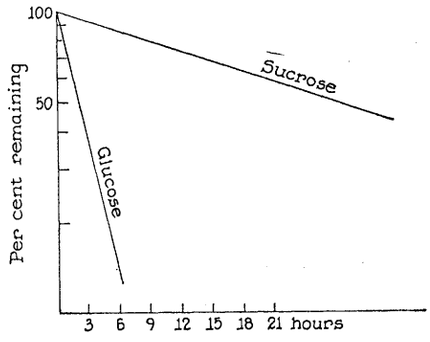

Fig. 1. Comparative escape rates of glucose and sucrose through a selective dialysis membrane.

If the concept of selectivity demonstrated in Fig. 1 is to be followed further, a method must be developed for adjusting the pore size to a rather critical range optimum for the particular size of the solute molecule. This can be accomplished (*8*) rather simply by controlled stretching, by controlled acetylation to reduce pore size, or by controlled swelling through treatment with a strong $ZnCl_2$ solution. Any pore size—from the smallest, which will reject amino acids, to the largest, which will readily pass solutes of molecular weight 100,000—can be produced at will. Once a membrane has been adjusted to a desired pore size it can be calibrated and used repeatedly until it fails mechanically.

With this brief statement of the problem, of the objectives, and of the approach to be used, we pass to a

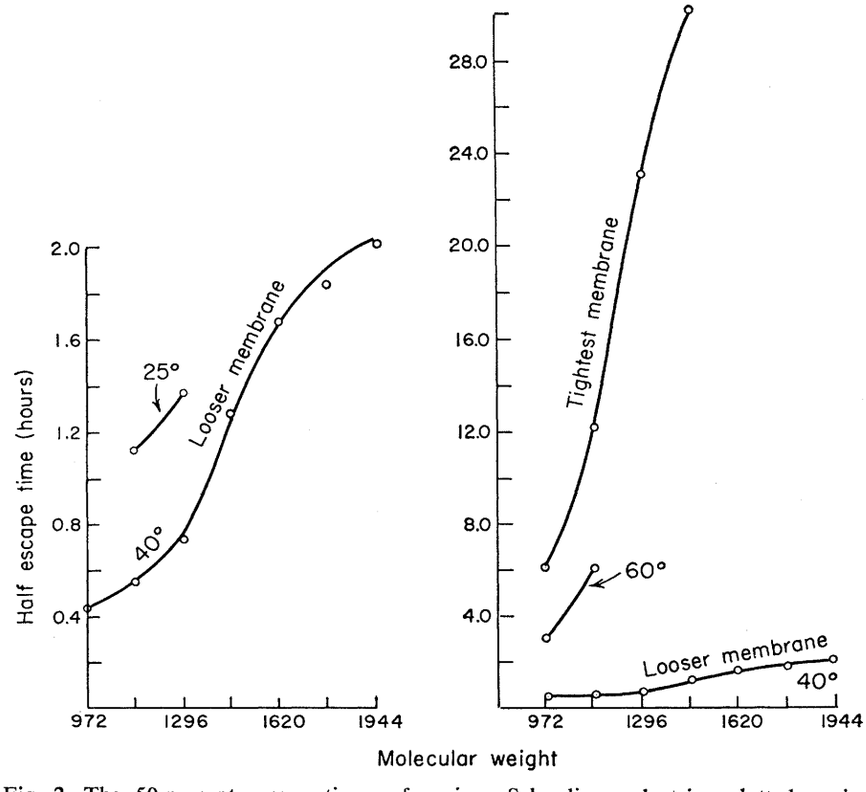

Fig. 2. The 50-percent escape times of various Schardinger dextrins plotted against their molecular weights.

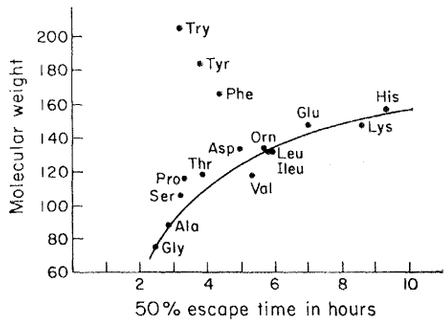

Fig. 3. The 50-percent escape times of various amino acids plotted against their molecular weights.

consideration of some of the data thus far accumulated with solutes of known molecular size and shape. Sugars are excellent models, since much is known about their size and conformation. Different dialysis rates (*10*) were found with different isomeric pentoses, hexoses, and disaccharides when the comparisons were made with membranes affording 50-percent escape times of a few hours.

Cyclic Polymers

Perhaps the best series of models for testing the approach to selectivity in differential dialysis is to be found in the Schardinger dextrins (*13*). These are rigid cyclic polymers of glucose whose molecular size and shape have been determined by x-ray diffraction studies. Six members of the series from cyclohexaamylose to cyclododecaamylose were studied (*10*). Some of the results are shown in Fig. 2.

These models are doughnut-shaped molecules approximately 7.8 angstroms thick, with the diameter of the ring approximately 13.7, 15.3, and 16.9 angstroms, respectively, for the hexa-, hepta- and octa- homologs. There is thus an increase in diameter of about 11 percent from one homolog to the next. From the data in Fig. 2 it may be seen that this increase in size is sufficient to decrease the dialysis rate by a factor of 2 when the 50-percent escape time of the cyclohexaamylose is 6 hours. On the other hand, a more porous membrane with a 50-percent escape time of 0.42 hour shows little selectivity: 0.42 and 0.55 hour, respectively, for the hexa- and hepta-homologs.

If the implications to be drawn from Fig. 2 are valid generally, then this type of differential dialysis should afford a way of amplifying the differences noted in free-diffusion studies. Further support for this conclusion is to be found in the data (*10*) given in Table 1. The free-diffusion data are those of Longsworth (*14*). Here it may be seen (cols. 4 and 6) that the ratios of free-diffusion rates are, in every case except one, greatly amplified by thin-film-membrane diffusion. The degree of amplification is a function primarily of the porosity of the membrane, but other factors may well have some influence.

Amino acids are interesting models because here the charge effect is relatively high compared to the molecular size. In spite of this, diffusional size is the parameter which determines the relative escape rate, as may be seen from the data (*15*) of Fig. 3. Obviously, cellophane treated by acetylation to decrease the pore size must be practically free of carboxyl groups. Direct potentiometric measurement supported this conclusion. The rate of dialysis can therefore be correlated with molecular size in spite of a fixed charge on the solute. However, with the aromatic amino acids a degree of adsorption to the membrane was observed. This seemed to cause them to show a diffusion rate somewhat higher than that expected. This effect was observed many years ago by Sollner (*16*) in his studies with the membranes available at that time. Such an effect becomes much smaller when the aromatic amino acid is part of a long polypeptide.

Table 3. Comparative dialysis rates of selected hormones (*45*).

Peptide	Molecular weight	$T/2$ (hr)* In 0.01*N* AcOH	$T/2$ (hr)* In 0.15*N* NH_4Ac, 0.01*N* AcOH
Angiotensin II amide	1031	3.6	
Oxytocin	1007	3.2	3.6
Deamino oxytocin (*46*)	992	3.5	
Lysine vasopressin	1056	5.3	5.5
Deamino lysine vasopressin (*47*)	1041	3.1	3.6

* $T/2$, 50-percent escape time.

Many different polypeptides have been studied (*17, 18*) with the thin-film technique. It has been found that while homologous series of peptides show the differential dialysis rates expected on the basis of their molecular weight, considerable differences can be noted when there is substitution of certain amino acid residues. Presumably this difference reflects an alteration in conformation, since association as a cause has been eliminated by studies in the ultracentrifuge.

Moreover, certain peptides show a considerably altered rate of dialysis when a salt such as ammonium acetate is added to the solvent. It has been found that this altered rate apparently can be correlated with the distribution

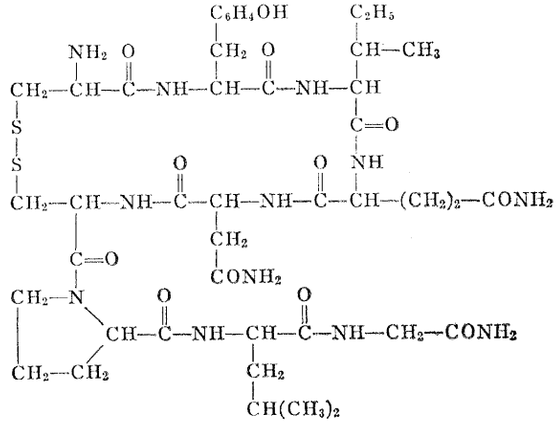

Fig. 4. Formula of oxytocin.

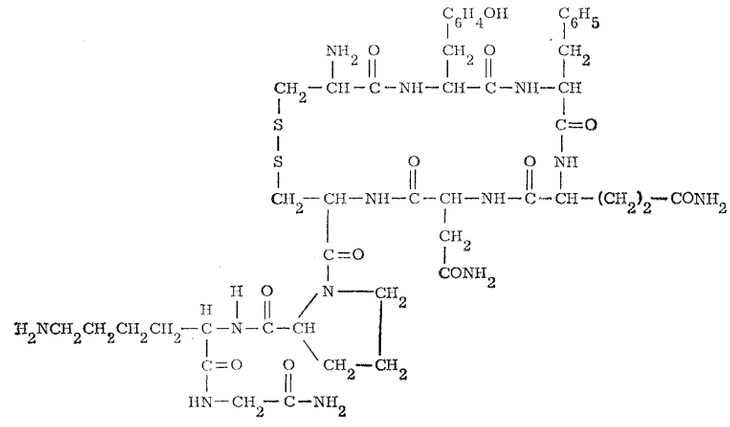

Fig. 5. Formula of 8-lysine vasopressin.

of charge on the peptide (*17*). Peptides in which the opposing positive and negative charges are separated from each other by at least several amino acid residues are likely to have their dialysis rate markedly retarded by the addition of salt, but peptides in which the opposing charges are close together are not influenced by the salt. This is consistent with the theory that the distribution of charge plays a strong role in fixing the shape of the molecule. In illustration of this point, Table 2 presents some of the data found with a series of peptides isolated from a tryptic digest of the chains isolated from hemoglobin (*19*).

Peptides in which the conformational possibilities are greatly restricted by peptide ring formation are of special interest as models in the study of differential dialysis. In these, as compared to linear peptides, considerably smaller differences in structural detail are apparently detectable by dialysis. This might be expected on the basis of the likelihood that linear peptides in solution really do not have a fixed conformation but present a statistical average of many interchanging conformations. Thus, oxytocin (*20*) (Fig. 4) and vasopressin (*21*) (Fig. 5) are of considerable interest. The ring structure is of such size that it must be of fixed conformation. However, the side chain could be either extended, to give the most asymmetric conformation possible, or attracted by secondary forces (hydrogen bonding, hydrophobic bonds, and so on), to present a three-dimensional shape more nearly spherical in form. In any case the data of Table 3 were found; they seem to indicate clearly that vasopressin has a larger diffusional size than oxytocin. Therefore, in vasopressin the side chain must be more extended away from the ring. Vasopressin has a basic group on the lysine residue in the side chain which would cause it to be repelled by the amino group on the cysteine residue. This interpretation is supported by the finding that removal of the basic amino group on the cysteine residue gives a peptide which diffuses at nearly the same rate as oxytocin or deamino oxytocin. We can thus reason that oxytocin must have a conformation of near-minimal diffusional size. Since angiotensin II amide,

$Asp{NH_2}$-Arg-Val-Tyr-Val-His-Pro-Phe,

with nearly the same molecular weight, dialyzes only a little more slowly, it too must have an effective conformation

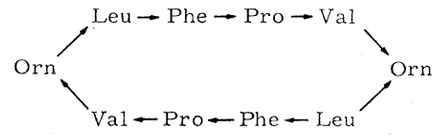

Fig. 6. Formula of gramicidin S.

approaching that of a rather tightly packed sphere.

Gramicidin S (*22*) (Fig. 6) is a well-characterized cyclic peptide model which has been synthesized (*23*). The dimensions of the unit cell in the crystal have been determined by x-ray diffraction studies (*24*). They are approximately 19 by 11 by 5 angstroms, but these are not necessarily the dimensions of the molecule in solution. Schwyzer (*23*) has suggested a conformation consistent with these dimensions, but Warner (*25*) has postulated a somewhat different one. In surface-film studies (*26*) it has been found that gramicidin S spreads on water to form a very stable solid type of film easily transferable to a slide. One side of the film is strongly hydrophobic, the other, hydrophyllic. In spite of the high stability, the thickness of the film is only 5 angstroms. The peptide ring thus lies flat on the surface. Data obtained with cyclohexaamylose (Table 4) in the same membrane indicated that the two ring compounds are of almost the same molecular size. This finding is entirely consistent with the x-ray data.

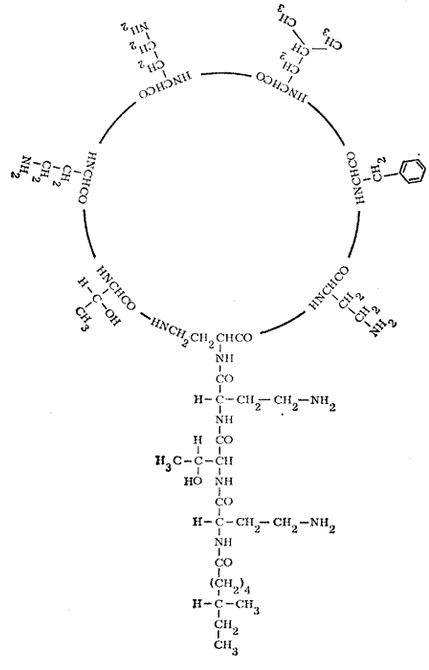

Fig. 7. Formula of polymyxin B_1.

When compared to the oxytocin-vasopressin group by dialysis in 0.01*N* acetic acid, gramicidin S was found to require a considerably more porous membrane. Further, when gramicidin S was compared to another well-characterized cyclic peptide, polymyxin B_1, whose most probable structure (*27, 28*) is shown in Fig. 7, it was found that the latter dialyzed at approximately half the rate at which gramicidin S did (Table 4). Polymyxin B_1 has no acidic groups but carries five strongly basic amino groups distributed around the molecule. In the charged state these would be expected to repel each other. Since there are no structures in the molecule with strong mutual attracting capabilities it seems reasonable to conclude that this peptide has an extended conformation, perhaps as fully extended as the covalent linkages will permit. Removal of the side chain enzymatically (*28*) gives a cyclic peptide dialyzing at four times the rate of the peptide with the side chain. Even so, this peptide dialyzes a little more slowly than does angiotensin I, in spite of the fact that it is a heptapeptide with molecular weight of 762. Gramicidin S, also with no carboxyl groups, has only two basic groups and may have a somewhat more condensed structure than polymyxin B_1, due to hydrogen bonding, as Schwyzer (see *23*) has suggested. Preliminary studies (*29*) made by means of deuterium exchange have indicated that the hydrogens in question are rather easily replaced.

Bacitracin (*30*) is also a particularly interesting model for these studies. It is known to have a covalent structure closely approximating that shown in Fig. 8. However, there is good chemical evidence to indicate that the Ileu-Cys-Leu tail is not extended but is held to the phenylalanine residue of the ring in some way. If this is the case, this would result in a conformation not far from a spherical shape. This concept is supported by dialysis. Bacitracin (Table 4), even though it is of larger molecular size and a dodecapeptide, was found to dialyze more rapidly than gramicidin S. All four peptides and cyclohexaamylose behaved almost ideally in the dialysis studies. In addition, ultracentrifuge data indicated the absence of significant association. The conformation of bacitracin, as evidenced by dialysis studies, was little influenced by salt and urea, another indication of a rather fixed and rigid structure.

Linear Polymers

At this point it would seem desirable to have as a model for study a water-soluble linear polymer of appropriate length which would unquestionably exist in solution as a so-called "random coil." Two extremes of this model, arising from differences in solvent influence, could be considered. In one, the balance of secondary forces along the chain could favor intrachain attraction and tend to cause random folding or coiling. This would result in a more globular shape and a smaller diffusional size. In the other, there would be no intrachain interaction, and a maximum diffusional size would be expected.

Polymers of ethylene glycol come to mind in connection with the latter extreme. They are water-soluble and are available commercially from the Carbon and Carbide Chemical Company. While strictly monodisperse polymers are not sold, polymers of rather narrow ranges of molecular size are available, called "Carbowax" 1000, 1520, and so on. These proved very interesting, although, as expected, the escape patterns were plainly curved, in contrast to the peptide and Schardinger dextrin patterns, which were all straight lines. Both Carbowax 1000 and Carbowax 1520 had escape rates considerably lower than those of most of the peptides and sugars of similar molecular weight. A comparative value of 2.4 hours for Carbowax 1000 is given in Table 4. Its range of molecular weight is given by the manufacturers as 950–1050. This escape rate contrasts with 0.95 hour for the linear decapeptide angiotensin I,

Asp-Arg-Val-Tyr-Val-His-Pro-Phe-His-Leu

of molecular weight 1282 (*31*). Obviously, angiotensin I must be rather tightly coiled or folded in solution, although the coil could well be a random

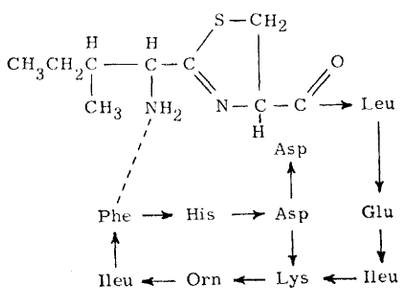

Fig. 8. Formula of bacitracin A.

one. In fact, angiotensin I probably exists in a state of equilibrium between many different forms, some of which are more extended than others. Angiotensin I has amino and carboxyl groups, which would favor a more compact form.

Still another example is obtained when the ring of tyrocidin B, a cyclic decapeptide, is split at the -Phe-Pro- peptide bond by reduction (*32*). A linear decapeptide,

Pro-Try-Phe-AspNH$_2$-GluNH$_2$-
Tyr-Val-Orn-Leu-Phe,

is formed which has the carboxyl group of the terminal phenylalanine reduced to hydroxyl. It thus has no carboxyl but has two basic groups, the imino group of the proline and the amino group of the ornithine. The rate of dialysis given in Table 4 for this decapeptide indicates that its structure is considerably more extended than that of angiotensin I. Tyrocidine B itself, before reduction, would not diffuse through this membrane at an appreciable rate, because it is strongly associated. This property was shown by studies in the ultracentrifuge as well as by dialysis.

With these studies as a background, data obtained with longer linear polypeptides have become more meaningful. Examples of these are the synthetic 23-amino-acid fragment of the ACTH molecule (*33*); porcine ACTH, with 39 amino acid residues (*34*); and parathyroid hormone (*35*), with approximately 70 residues. These peptides gave dialysis rates in 0.01*N* acetic acid that were close to what would be expected on the basis of their molecular weight, but in each case the rate was markedly retarded when ammonium acetate was added to the solvent. The retardation, which ranged from 10-fold to 15-fold, was shown by sedimentation studies on ACTH not to be due to association. Evidence too involved to present here has been accumulated which indicates that these linear peptides in salt-free water or 0.01*N* acetic acid are apparently coiled in some random way in a form approaching minimal volume. Opposing positive and negative charges strategically located along the peptide chain probably play a strong role, since with these particular peptides the addition of salt has such a striking effect on the dialysis rate. Salt would be expected to depress the constricting effect of the opposing charges.

Ribonuclease is a good model of a polypeptide of larger size (*36*). It has a molecular weight of 13,600. According to a recent estimation (*37*), the molecule, in solution, approximates a short cylinder 22 angstroms in diameter and 66 angstroms long. It could be more spherical in certain solutions, and indeed ribonuclease has been found to dialyze more rapidly in 0.01*N* acetic acid than in 0.15*N* salt solution (*38*). Lysozyme (molecular weight, 15,000), chymotrypsinogen (molecular weight, 24,500), pepsinogen (molecular weight, 42,000), and serum albumin (molecular weight, 66,000) have been studied (*38*) by the dialysis method, and the rates of dialysis have been found to be consistent with published data relative to their molecular sizes. The effect of salt and urea has also been investigated.

The behavior of the transfer ribonucleic acids in dialysis is particularly interesting. They are known to have a molecular weight in the range of 30,000 (*39*). In contrast to findings for most proteins of this size, the presence of salt in the solution makes them diffuse more rapidly (*39*) through cellophane membranes of appropriate porosity. Since they are polyanions, the shielding effect of the salt might be expected to permit a more condensed molecular conformation. A similar effect was noted with the A chain from the oxidation of insulin. Here there is one terminal basic group, but there are, in all, seven carboxyl and sulfonic groups distributed along the chain.

Solutes that are known to associate strongly or to exist as association complexes have been studied. These include the tyrocidines, insulin (*40*), and hemoglobin (*41*). Each of these has been found to show characteristic behavior during dialysis.

Gel Filtration

In recent years a very useful method, called "gel filtration," for separating mixtures has been developed by Flodin (*42*), Porath (*43*), and their associates. The procedure is very similar to chromatography, but the separation seems to depend mainly on the effective diffusional size of the solute. Adsorption often plays a role, however, particularly when the solutes contain aromatic ring structures. In spite of this, the results often correlate well with those of thin-film dialysis. Gel

Table 4. Comparative dialysis rates of selected solutes of known structure.

Solute	Molecular weight	$T/2$ (hr)*
Carbowax 1000	950–1050	2.4
Angiotensin I	1282	0.95
Tyrocidine B (ring split)	1348	2.1
Cyclohexaamylose	972	2.1
Polymyxin B_1	1208	5.6
Ring fragment of polymyxin B_1	762	1.4
Gramicidin S	1142	2.4
Bacitracin	1420	1.8

* $T/2$, 50-percent escape time.

filtration is not discussed further here, since it was thoroughly reviewed recently in *Science* (*44*).

The results obtained with gel filtration, together with the surprisingly high selectivity obtainable with simple differential dialysis, suggest that size or conformation (or both) may play a far greater role in the selectivity of all chromatographic or zone electrophoresis processes than has heretofore been thought. The marked change in dialysis rates found, for many solutes, to accompany small changes in *p*H, small changes in salt content of the solvent, or relatively small changes in temperature gives interesting grounds for speculation. Such effects could well play a role in any separation process where there is restricted diffusion through porous material.

Future Possibilities

It seems of some interest to extrapolate from the limited results given here and to speculate on the selectivity obtainable if retentate films of the order of 0.01 millimeter or less in thickness could be produced and used experimentally, with membrane areas ten times greater than at present. This would make it possible to achieve a much more critical adjustment of pore size. (It may be pertinent to point out that such thin solution films and relatively enormous membrane surfaces seem to occur in living tissues.) The overall rates of diffusion are high because of the short distances the solutes need travel. Any change in pore size would be expected to be extremely critical and could be brought about by a slight mechanical stretching or by specific salt effects. The conformation of many polypeptides apparently is greatly influenced by specific salt effects, and the possible implications of this property, if polypeptide material should form part of a membrane, seem obvious. The great selectivity of the charged membranes existing in living tissues has been known for a long time and has been much studied. It is no surprise to find that some of the same problems are ultimately encountered when one begins by asking the question "Is it dialyzable?"

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