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Passage of Inulin and *p*-Aminohippuric Acid through Artificial Membranes: Implications for Measurement of Renal Function

E. Middleton

Department of Hormone Physiology, Imperial Cancer Research Fund, London, WC2A3PX, England

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Summary. Diffusion of inulin and *p*-aminohippuric acid (PAH) in combined aqueous solution through artificial membranes was measured at room temperature and atmospheric pressure. The membranes had pore diameters of 26, 50, 100, 200, 250, 350, 510 or 990 Å. The diffusion of PAH was only restricted with a pore size of 26 Å, but inulin diffusion was restricted at 100 Å. When diffusion of both solutes was unrestricted (pore diameter $\gtrsim$200 Å), PAH diffused four times faster than inulin, and in restricted situations this ratio was even greater. The results of these diffusion studies allow the major and minor molecular dimensions of the solutes to be estimated. Filtration of the two solutes was studied in slowly flowing situations and also with increased temperature and pressure. Pore sizes required for unrestricted filtration were the same as for unrestricted diffusion but the passage ratio was reduced from 4 to 2. These results suggest strongly that two conditions are necessary if the glomerular filtration rate (GFR) of inulin is to equal the true GFR: membrane pore size must be at least 200 Å and passage through the membranes must be by bulk transport.

Our interest in filtration of inulin and *p*-aminohippuric acid (PAH) arose from difficulties in interpreting some renal-function tests in hamsters with renal tumors – hence the present study of the diffusion or filtration of these substances through artificial membranes *in vitro*.

Inulin clearance is commonly used in man and laboratory animals as a measure of glomerular filtration rate (GFR); the renal clearance of PAH is generally greater than that of inulin and the difference between these clearances is commonly used as a measure of tubular secretion. This procedure is acceptable so long as both substances filter freely and at the same rate through glomerular membranes – otherwise not.

Presumably, the most important influence on the diffusion or filtration of a substance is the relation between its molecular size and that of the

membrane pores. In our studies the pore sizes used (26–990 Å diameter) ranged well beyond those currently believed to be present in the mammalian glomerulus (75–100 Å).

The manner in which the results are discussed reflects our interest in the implications for the measurement of renal clearance though the results may also be of general interest.

Materials and Methods

Membranes

The membranes used in these studies were obtained from three suppliers and covered a range of pore size (*see* Table 1).

Table 1

Pore diameter (Å)	Type of membrane
26	Dialysis tubing
50	Sartorius Hygrocella membrane filter
100	Sartorius Hygrocella membrane filter
200	Sartorius Hygrocella membrane filter
250	St. Mary's Hospital, London, Gradicol membrane
350	Sartorius Hygrocella membrane filter
510	St. Mary's Hospital, London, Gradicol membrane
990	St. Mary's Hospital, London, Gradicol membrane

The pore diameters are those quoted by the three manufacturers. Since the results of the present studies are coherent over the complete range of pore size (26–990 Å) these diameters are presumed correct.

Apparatus

Each membrane assembly consisted of a perspex tube 2 inches in diameter with a membrane at its base (Fig. 1, left). This was held in position by a vacuum "O" ring, the appropriate surfaces being lightly smeared with silicone grease. The complete assembly was secured with stainless steel springs.

In a first experiment the assembly was used in its simplest form: the perspex tube was open at the top and unattached to any other equipment. In two further experiments the tube was attached to a peristaltic pump (Fig. 1), and for this purpose the tube was capped with a perspex disc having two small outlets. Because the membrane was now to be subjected to pressure, it was supported by an open-mesh stainless steel grid.

Experiments

In all three experiments a combined solution of inulin and PAH of equal concentrations was placed in the perspex tube (the tank) which was lowered into a dish of distilled water (the sink). The complete unit was enveloped in Parafilm to minimize evaporation.

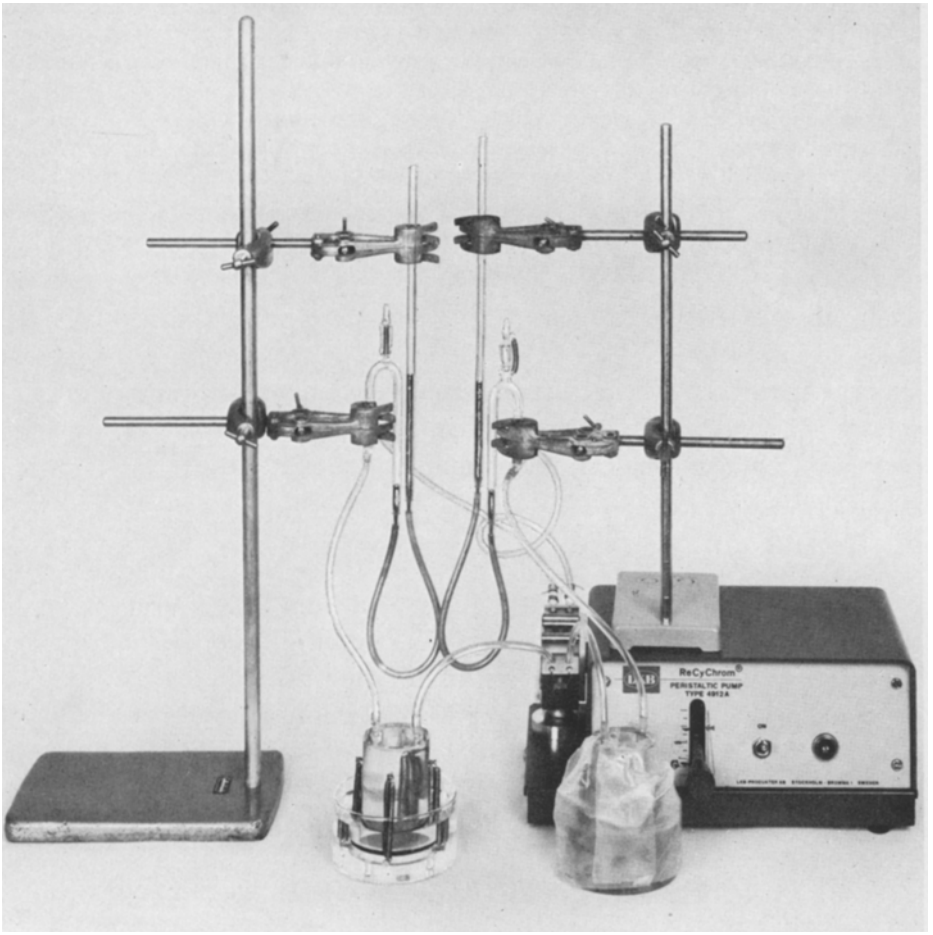

Fig. 1. Apparatus used for experiments

In Experiment 1, passage of the solutes through the membranes was wholly by diffusion; in other words, a simple exchange of solutes and water between tank and sink. This experiment was carried out with aqueous solution at room temperature and pressure, the meniscus in the tank (30 ml) being level with that in the sink (200 ml); most of the combinations of eight pore sizes and five solute concentrations (*see* Results) were studied. For each combination there were four tanks and sinks used at the same time to allow sampling at four time intervals (generally 1, 2, 4 and 6 hr from the start).

In Experiment 2, passage of the solutes through the membranes was mainly by filtration and partly by diffusion; in other words, water as well as solutes passed from the tank to the sink. In this experiment the aqueous solution was circulated through the tank by means of a pump, and excess pressure was exerted by a manometer; the flow circuit was duplicated to provide duplicate results. All combinations of two flow-rates *over* the membrane (1.5 and 12 ml/cm^2 hr), two concentrations (0.5 and 0.05%), two temperatures (21 and 37 °C), two pressures (atmospheric and atmospheric + 90 mm Hg) and four pore sizes (*see* Results) were studied.

Experiment 3 partially repeated Exp. 2 (concentration 0.05 and 0.15%, pressure atm + 90 mm Hg, temperature 37 °C, flow-rate 12 ml/cm² hr and pore size 50 Å) but used heparinized rabbit blood as solvent. We were not in the position to study filtration in situations of turbulent flow (*see* Discussion).

The solutions used in these experiments were heated to 60 °C, allowed to cool to the required temperature and used immediately. Inulin was estimated by the method of Bacon and Bell (1948) and PAH by the method of Bratton and Marshall (1939).

Results

In the review *The Physics of Porous Membranes* (Bean, 1972), the accepted unit of solute flow (J_s) is the mole/cm² sec; but since the present studies concern the measurement of renal function where clearance rates of solutes are expressed in terms of mass, and since here some of the flow-rates are extremely small, it seems more appropriate to express J_s as μg/cm² hr.

Experiment 1: Diffusion

It is not necessary to present the raw data from this experiment. In all situations the exponential decrease in the rate of diffusion of both solutes with time was small, so that the mean diffusion rates during the first two or three hours (Fig. 2) adequately represent the diffusion rates at time zero;

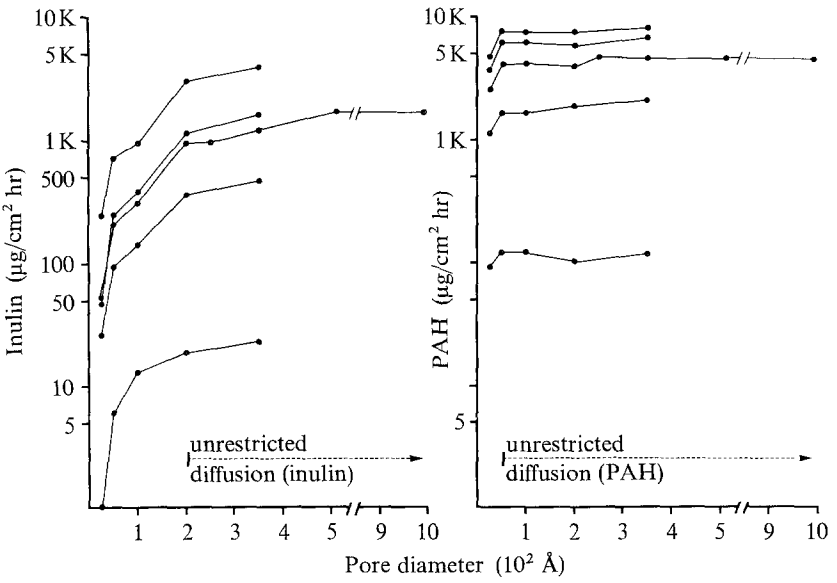

Fig. 2. Rate of diffusion of inulin (left) and PAH (right) as a function of pore diameter. For each solute the situation of unrestricted diffusion is indicated by a broken line. Note that the ordinate scale is logarithmic. The five curves for each solute represent (top to bottom) solute concentrations of 2.0, 1.5, 1.0, 0.5 and 0.05%

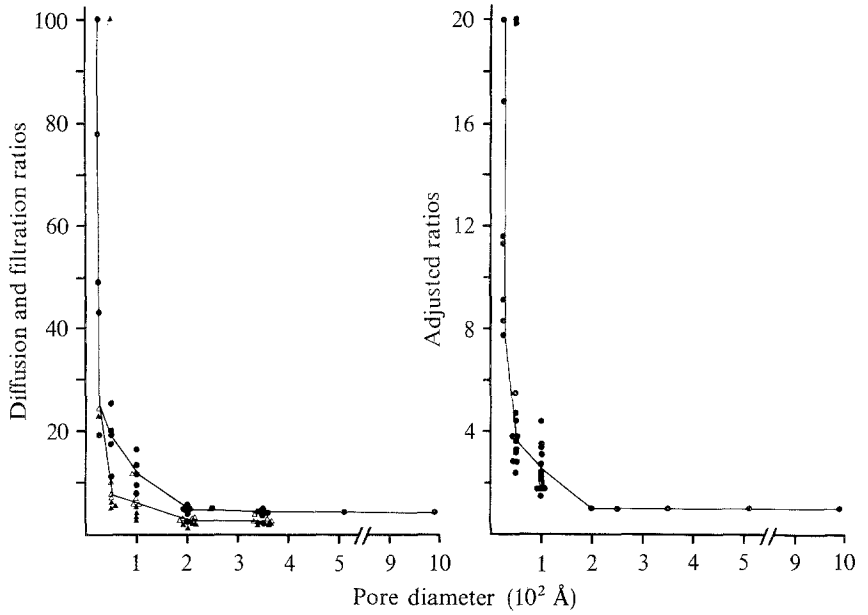

Fig. 3. *(Left)* PAH/inulin diffusion (upper curve) and filtration (lower curve) ratios as a function of pore size: •, diffusion; ▲, filtration at high pressure; △, filtration at low pressure. In the filtration plots no distinction is made between the two temperatures *(see text)*. For pore sizes 200 Å or greater, the ratio is 4.3 ± 0.3 (SEM, $n = 13$) for diffusion and 2.4 ± 0.1 (SEM, $n = 64$) for filtration. *(Right)* the above ratios adjusted to show only the effect of pore size *(see text)*. No distinction made between diffusion and filtration (*see* Table 3)

this is the only moment at which it is theoretically valid to compare the diffusion rates of PAH and inulin, for only here are conditions identical for both solutes.

The diffusion of inulin at any particular concentration increased with increasing pore size until the size of the pore allowed unrestricted diffusion. This occurred with pores of about 200 Å diameter (probably a little above this figure), but diffusion was certainly restricted with pore size of 100 Å.

In every situation PAH diffused much faster than inulin and, at any particular concentration, the diffusion rate was fairly constant when the pore size was 50 Å or greater. Diffusion of PAH was certainly restricted with pore size of 26 Å.

The difference between the diffusion rates of PAH and inulin is reflected in the diffusion ratios (Fig. 3, left). The ratio was fairly constant (4.3 ± 0.3, SEM) when the pore size was 200 Å or greater, but increased considerably and became more variable as pore size diminished. Most of this increased variability is associated with the steep slope of the graph in this area, though

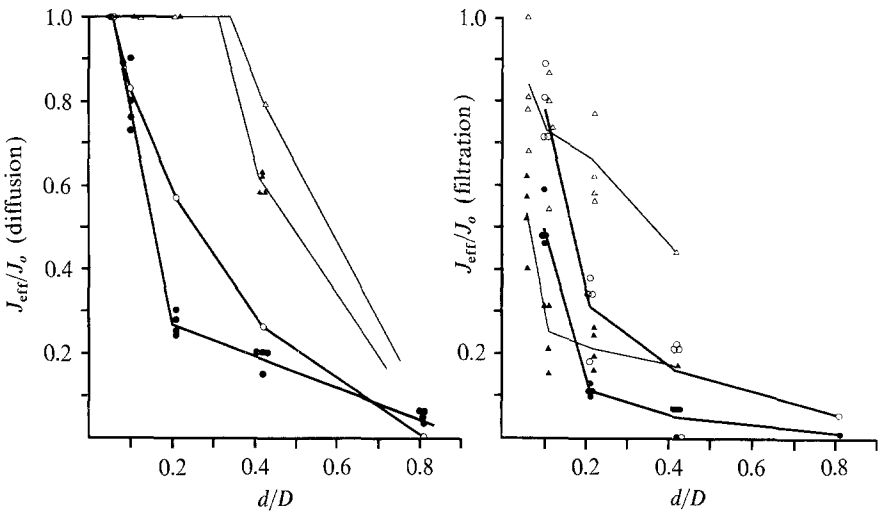

Fig. 4. The ratio between effective (J_{eff}) and unrestricted (J_O) passage as a function of the ratio between smaller molecular dimension (d) and pore diameter (D). *(Left)* diffusion: ●○, inulin (thick lines); ▲△, PAH (thin lines); open symbols, solute concentration 0.05%; solid symbols, solute concentrations $\lesseqgtr$0.5%. The inulin lines are fully defined but those for PAH depend on extrapolation, most of the values being on the horizontal part of the graph. *(Right)* filtration: symbols as above except, open symbols, low pressure; solid symbols, high pressure. No distinction made between the two temperatures or the two concentrations

some might be attributed to clogging of the small pores by the large inulin molecule.

If allowance is made (*see* Bean, 1972) for the ratios between pore size and molecular size on the one hand and between effective diffusion and unrestricted diffusion on the other, then the conclusions about the diffusion ratio of the two substances still holds. These relations are illustrated in Fig. 4 (left) where the smallest molecular dimensions (*see below*) are used.

A further aspect of diffusion apparent in this graph is that diffusion of either solute was relatively greater at the very low concentration (0.05%) than at the higher concentrations ($\lesseqgtr$ 0.5%). Perhaps we should note that possibly this method of plotting the results is invalid when considering diffusion (or filtration) of a nonspherical molecule such as inulin.

We do not know if these diffusion studies yield the true diffusion ratio for, although the diffusion coefficient for inulin is about 2.2×10^{-6} cm^2/sec (Bunim, Smith & Smith, 1937) apparently that for PAH is not known. However, Stein and Nir (1971) suggest that the diffusion coefficient decreases exponentially as the molecular weight of the diffusant increases; values in the literature support this claim and, by interpolation, the diffusion coeffi-

cient of PAH (mol wt 194) would be about 7 implying a PAH/inulin diffusion ratio of about 3.2 rather than 4.3, the value found in the present study. It seems that interposing any of the membranes used in these studies restricts the diffusion of inulin more than that of PAH.

The main conclusion from this experiment is that inulin does not diffuse freely through membranes of pore size less than 200 Å. In nonrestricting conditions (pore diameter $\geqslant 200$ Å) PAH diffuses four times faster than inulin; in restricting conditions (pore size < 200 Å), the difference is even greater.

Experiment 2: Filtration; Water as Solvent

In this experiment further variables were introduced: pressure, temperature and flow through the tank. Two rates of flow were used (1.5 and 12 ml/cm^2 hr) but there was no significant difference in filtration rates or ratios between the two under the same conditions of temperature and pressure. Thus, this factor has been ignored in Fig. 5 which gives the mean results for the two rates of flow. This does not mean that the imposition of flow had no effect – at room temperature and atmospheric pressure (i.e. under the same conditions as in Exp. 1) it reduced rates of filtration compared with Exp. 1, and to a much greater extent for PAH than for inulin (Fig. 6). It should be remembered that the flow rates were quite

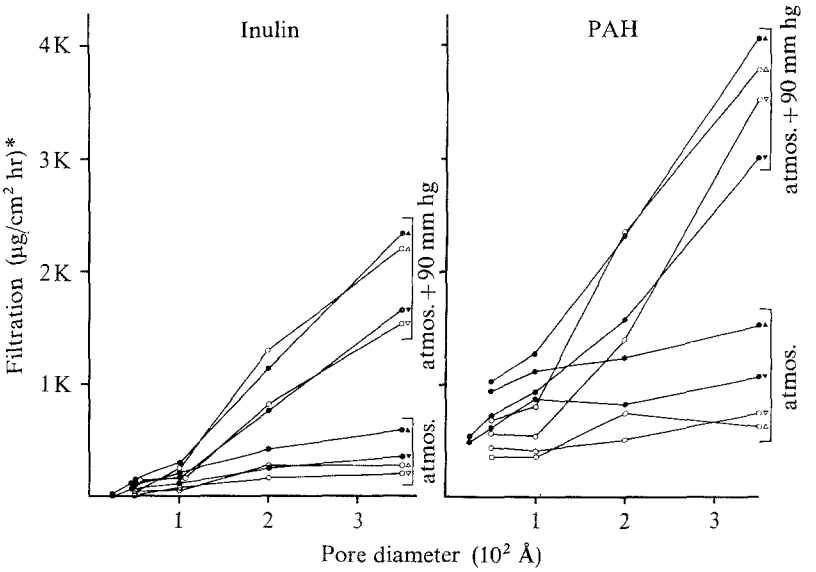

Fig. 5. Rate of filtration of inulin (left) and PAH (right) as a function of pore diameter: •, solute concentration 0.5% (ordinate scale as shown); ○, solute concentration 0.05% * (ordinate scale changed from 4 K to 0.4 K); ▲△, 37 °C; ▼▽, 21 °C

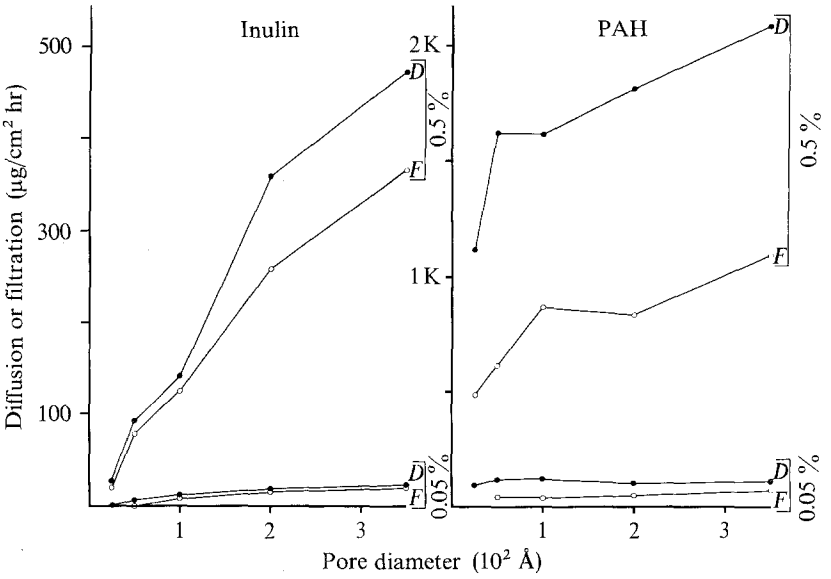

Fig. 6. A comparison of diffusion (Exp. 1) and filtration (Exp. 2) rates for inulin (left) and PAH (right) under conditions of room temperature and atmospheric pressure: •(*D*), diffusion; ○(*F*), filtration. The solute concentrations (0.5 and 0.05 %) are indicated

small compared with the volume of the tank (90 ml) and the situation is hardly comparable with glomerular conditions (turbulent flow).

Filtration was increased by increased pressure and/or temperature but the extent of the increase was governed by pore size. The increase produced by increased pressure was small with pore sizes < 100 Å and great with pore sizes of 200 and 350 Å. Raised temperature also increased filtration more with large pores than with small ones though the differences were not nearly so great as for pressure. When the filtration ratios are considered (Fig. 3, left) the different quantitative effects on the two solutes result in raised pressure reducing the ratio but raised temperature having little effect.

These effects of raised pressure are also apparent (Fig. 4, right) where the same relation is plotted for filtration as has already been plotted for diffusion (Fig. 4, left). Unlike the situation with diffusion, this relation was not markedly different either between the two solutes or between the two concentrations (0.05 % and 0.5 %). This latter finding is similar to one by Renkin (1954): he found that molecular sieving of sucrose is independent of concentration.

The demarcation between restricted and unrestricted filtration is not clear as it was for restricted and unrestricted diffusion in Exp. 1, but when the filtration ratios are calculated (Fig. 3, left) the line of demarcation is

exactly the same in the two experiments and clearly, in comparison with the filtration of PAH, that of inulin is restricted when the pore size is less than 200 Å. However, the filtration ratios themselves were generally lower than the diffusion ratios by about 50%; apparently creation of flow *over* the membranes restricts PAH passage more than inulin passage.

Experiment 3: Filtration; Blood as Solvent

In this experiment (50 Å membrane only) heparinized rabbit blood was used as the solvent instead of water. The results of this experiment are only strictly comparable with one situation in Exp. 2 (50 Å membrane, 0.05% solution, 37 °C and high pressure), but to give a broader view of events the results of the present experiment using a concentration of 0.15% and those of Exp. 2 using a concentration of 0.5% are also included in Table 2.

It is fairly clear from this Table that filtration of both solutes was generally less in the present experiment than in Exp. 2, just as filtration in Exp. 2 was less than diffusion in Exp. 1. This was especially so for PAH so that the filtration ratios were lowered still further. The very low, and possibly inaccurate, values for the filtration rate of inulin at the low concentration (0.05%) and pore size (50 Å) clearly account for the large variation in estimates of the filtration ratio (∞, 8.3), but this does not invalidate the main conclusion from these studies that no matter whether blood or water is used as solvent, or whether the solutes are allowed to diffuse or are forced to filter through the membranes, the passage of inulin is restricted when the pore size is less than 200 Å.

Table 2. Comparison of filtration rates and ratios in Exps. 2 and 3 [a]

Exp.	Solute concentration (%)		
	0.05	0.15	0.5
	μg inulin/cm² hr		
2	0	(38)	173
3	6	27	–
	μg PAH/cm² hr		
2	71	(286)	1039
3	50	129	–
	PAH/inulin ratio		
2	∞	(7.5)	6.0
3	8.3	4.8	–

[a] 50 Å membrane, 37 °C, high pressure, high flow-rate. Values in parentheses obtained by interpolation.

Dimensions of PAH and Inulin Molecules

The diffusion studies (Exp. 1) allow estimation of the size of both molecules in terms of their major and minor axes. When a molecule is more elliptical than spherical, diffusion is governed by the major axis rather than the minor axis, though the minor axis determines the minimum pore size through which a molecule can pass. According to Wesson (1969), when the radius of a (spherical) molecule exceeds 25% of the pore radius, the molecule experiences difficulty in entering the pore (steric hindrance) and tends to be restricted (molecular sieving). The present studies indicate that this occurs at a pore diameter of 200 Å for inulin and 50 Å for PAH, so that apparently the major axis of the inulin molecule is about 50 Å, and that of PAH about 12 Å.

The length of the minor axis of each molecule can be estimated with some confidence from the results of Exp. 1 (Fig. 2). If, for every solute concentration, the diffusion rate is plotted against membrane pore size, the resulting curves have a common point of intersection at the x-axis defining the situation where diffusion ceases ($y = 0$) because the membrane pore size equals the length of the minor axis of the molecule.

An estimate of this dimension can be obtained by deriving "least-squares" quadratic equations which relate the diffusion rate y to the membrane pore size x at the various concentrations, and then finding their mean point of intersection with the x-axis. The derivation of a quadratic equation requires at least three pairs of coordinates, and for inulin suitable values are available at $x = 26$, 50 and 100 Å.

From the nature of the PAH results it is clear we lack an observation between 26 and 50 Å and cannot safely interpolate between these points. However, within this range we can safely interpolate for the inulin diffusion rate (Fig. 2, left) and for the diffusion ratio (Fig. 3, left); the PAH diffusion rate being the product of these can then be found for a third pore size (i.e. 35 Å).

The equations relating the diffusion rate y to the membrane pore size x were invariably of the form $y = a + bx - cx^2$. The magnitudes of the coefficients a, b, c, were governed by the type of solute and the solute concentration: they were larger at the higher concentrations and were particularly large with PAH compared with inulin. Estimates of the length of the axes from these studies are for inulin: minor axis 21 ± 1 Å (SEM) and major axis c 50 Å; and for PAH: minor axis 11 ± 1 Å and major axis c 12 Å.

Previous estimates of the axial ratio of the inulin molecule vary from 5 (Galey & Van Bruggen, 1970) to 7–10 (Phelps, 1965); the present studies

indicate a much lower value of about 2.5. We are unable to vindicate this low value, but note that our estimates of the dimensions of the molecule (50×21 Å; ellipsoidal volume 11 545 cu Å) are compatible with the view that the inulin molecule behaves as a sphere with diameter 30 Å (Galey & Van Bruggen, 1970) and that it consists of about 35 fructose molecules each with dimensions $12 \times 3.5 \times 3$ Å (Phelps, 1965) and an ellipsoidal volume of 66 cu Å.

Discussion

Physical Considerations

Although many aspects of the complex kinetics of passage through membranes have been extensively studied (Renkin, 1954; Kedem & Katchalsky, 1958; Katchalsky & Kedem, 1962; Galey & Van Bruggen, 1970; Dietschy, Sallee & Wilson, 1971; Gary-Bobo & Solomon, 1971; Starzak, 1973; Stender, Kristensen & Skadhauge, 1973; Winne, 1973), the present state of the art does not seem to allow a strict mechanistic interpretation of the present results. This arises from the fact that theory and experiment seem to have been concerned with the diffusion and filtration of near-spherical molecules rather than with asymmetrical molecules such as inulin. Furthermore, in an interesting review on *The Physics of Porous Membranes*, Bean (1972) finds that theory for fine pores – where pore and molecular sizes are comparable – is somewhat underdeveloped. Despite this situation, some of the effects observed here can usefully be discussed in nonmathematical terms, particularly where they concern the diffusion and filtration ratios, which are dimensionless.

The main findings from the present studies are clear: passage of inulin through artificial membranes, whether by diffusion or filtration is restricted when the pore size is less than 200 Å, whereas PAH passes freely through membranes with pore size as small as 50 Å.

The situation in conditions of turbulent flow *over*, and bulk transport through, membranes was not studied but we suggest that the present results can be adjusted to illustrate this situation. Fig. 3 (right) and Table 3 show the values for the diffusion (Exp. 1) and filtration (Exp. 2) ratios after division by the mean ratio (approximately 4.3 in Exp. 1 and 2.4 in Exp. 2) for pore sizes $\lesssim 200$ Å in each situation. This calculation assumes that differences between coefficients of diffusion, though paramount in quiescent situations, are less important in slowly flowing situations and unimportant in turbulent situations. This rather drastic adjustment leads to the conclusion that inulin can filter at the same rate as PAH only if the membrane pore size is at least 200 Å and passage of the solutes is by bulk transport.

Table 3. PAH/inulin diffusion (Exp. 1: upper) and filtration (Exp. 2: lower) ratios adjusted to show only the effect of pore size (*see* text for details)

Solute conc. (%)	Temp. (°C)	Pressure (mm Hg)	Membrane pore diameter (Å)							
			26	50	100	200	250	350	510	990
			PAH/inulin ratio (adjusted)							
0.05	21	atm	∞	3.8	1.8	1.0	–	1.0	–	–
0.5	21	atm	9.1	3.6	2.4	1.1	–	0.9	–	–
1.0	21	atm	11.3	4.4	3.1	1.0	1.2	0.9	1.0	1.0
1.5	21	atm	16.9	5.5	3.5	1.1	–	0.9	–	–
2.0	21	atm	8.3	4.7	3.4	1.1	–	0.9	–	–
0.05	21	atm+90	–	2.8	1.8	0.8	–	1.2	–	–
0.05	21	atm	–	∞	1.5	0.9	–	1.1	–	–
0.05	37	atm+90	–	∞	1.8	1.0	–	1.0	–	–
0.05	37	atm	–	3.8	4.4	1.1	–	0.9	–	–
0.5	21	atm+90	11.6	3.3	2.7	1.1	–	0.9	–	–
0.5	21	atm	7.7	2.4	2.2	1.0	–	1.0	–	–
0.5	37	atm+90	–	3.2	2.1	1.1	–	0.9	–	–
0.5	37	atm	–	2.8	2.0	1.1	–	0.9	–	–
						Conditions for free passage of inulin and PAH (turbulent flow, bulk transport)				

The apparently high PAH/inulin diffusion ratio in Exp. 1 and low filtration ratio in Exp. 2 seem to indicate that interposing any of the membranes used here restricts inulin passage more than PAH passage, but that creation of flow *over* the membrane has the reverse effect.

The first effect can be explained if we accept that the frictional coefficient between solute and membrane (f_{sm}) is going to be greater for inulin than for PAH: the studies of Kaufmann and Leonard (1968) indicate that this coefficient increases greatly as solute molecular weight increases.

The second effect is more difficult to explain and the simplest explanation is that the smaller and lighter PAH molecule was more easily swept along in the current *over* the membranes than was the inulin molecule, thereby tending to remain in circulation longer than expected, though PAH still filtered at a greater rate than did inulin.

The filtration rates of PAH and inulin observed in Exps. 2 and 3 were lower than the diffusion rates in Exp. 1 and the simplest explanation for this is that in the first situation appreciable volumes of water passed through the membranes in the same direction as the solutes, especially at the higher pressure (about 0.01–0.5 ml/cm^2 hr depending on pore size), and that the

filtering capacity of the membranes was therefore shared between three instead of two substances. This was not the situation in Exp. 1 which was one of simple exchange of solutes and water between tank and sink, the actual volumes of water involved being minute.

Though there was a clear demarcation between restricted and unrestricted diffusion, and diffusion relative to pore size was less restricted at the very low concentration (0.05%) than at the higher concentrations, this was not so with filtration. This seems to imply that the resolving power of separation procedures is greater with diffusion than with filtration, though it should be noted that in the present studies the bulk fluid above the membranes was stationary in the experiments on diffusion whereas in those on filtration it was flowing *over* the membranes.

Renal Implications

We would now like to consider the results of the present studies insofar as they may concern the measurement of renal clearance. The situation *in vitro* is not strictly comparable with that in the glomerulus. For instance, Richardson, Ľicko and Bartoli (1973) find that the behavior of a folded membrane is quite different from that of a plane membrane; nevertheless, the restricting effect of a membrane of small pore size on the filterability of inulin must be common to all types of systems and we propose that in this respect, the glomerular situation lies somewhere between filtration (Fig. 3, left) and bulk transport (Fig. 3, right).

There are two views as to the importance of diffusion in glomerular passage: Hendrix, Westfall and Richards (1936) think that differences between coefficients of diffusion are unimportant factors in glomerular filtration whereas Chinard (1952) thinks otherwise. Perhaps both views are correct depending on the pore size and mode of passage: with large pores and bulk transport Hendrix *et al.* (1936) are correct; otherwise, Chinard (1952) is correct (*see* Fig. 3, right and left).

It has been clearly established by micropuncture studies of glomerular capsular fluid that inulin filters freely (unrestricted) through the glomerular membranes of amphibia (Hendrix *et al.*, 1936; Bott, 1952; Giebisch, 1956) and it therefore seems probable that in these species the effective glomerular pore size is at least 200 Å and glomerular filtration is by bulk transport.

This estimate of pore diameter in amphibia, derived from experimental studies, is considerably higher than that (64 Å) derived from mathematical functions which relate filtration, molecular size and pore size (*see* important review on glomerular filtration by Renkin and Gilmore, 1973). We are unable to vindicate our higher value but note an interesting aspect of the

lower value: the experimental results tabulated by Renkin and Gilmore (1973) show that for a protein of any given Stokes-Einstein radius in the range 19–53 Å, considerably more protein appears in the glomerular filtrate of amphibia than in that of mammals. Taken at face value this result seems to indicate that the effective glomerular pore size is considerably greater in amphibia than in mammals, though the authors find that the mathematical functions indicate a smaller pore diameter in amphibia than in mammals (64 Å against 72 Å).

The large discrepancy between our estimate of effective glomerular pore diameter in amphibia ($\leqslant 200$ Å) and that of Renkin and Gilmore (1973) (64 Å) remains to be explained if, in the present studies, the membrane pore sizes quoted by the three manufacturers are correct. Possibly, present theory is not sufficiently developed to deal with a situation of actual or near bulk transport as against filtration and/or diffusion: Renkin and Gilmore (1973) point out that ultrafiltration theory is based on crude macroscopic models of restriction.

Another likely source of error in using present theory to estimate pore size is that estimates are then based on the Stokes-Einstein radius of the reference molecule rather than on its molecular configuration. Here it is of interest that Renkin and Gilmore (1973) suggest that where clearances of substances of identical molecular weight are unequal, the differences are probably accounted for by differences in molecular configuration; the present studies clearly indicate the importance of this factor.

If the situation is unclear in amphibia, it is doubly so in mammals since no one has demonstrated *directly* that inulin filters freely through the glomerulus in these species. Although Walker, Bott, Oliver and Macdowell (1936) have studied glomerular capsular fluid in the guinea pig, and Lassiter, Gottschalk and Mylle (1961) have studied this fluid in the rat, neither group of workers dealt with inulin in glomerular fluid.

The belief that inulin filters freely through the mammalian glomerulus depends mainly on the finding that the clearance of inulin is independent of its molecular weight in man (Walser, Davidson & Orloff, 1955; Mogensen, 1968) and rabbit (Jørgensen, Møller & Sheikh, 1972). However, the effect of changes in molecular weight with a constant pore size is probably the same as varying the pore size with a constant molecular weight. Our results in the latter situation, where there is an exponential relation between the two factors, indicate that changes in molecular weight only noticeably affect filtration rate if the molecules are small. In other words, had inulin of low and high molecular weight been used separately in our studies the differential between inulin and PAH would have remained much the same.

Table 4

Substance	mol wt	GFR
Polyethylene glycol	400	1.12
Polyethylene glycol	1000	1.03
Polyfructosan	2760	0.94
Inulin	6000	0.78

Furthermore, this indirect evidence for the free filtration of inulin through mammalian glomerular membranes is at variance with other considerations of glomerular filtration: Wesson (1969) points out that if the filtration-coefficient studies of Pappenheimer (1955) are valid, then the pressure available to force water from plasma (25–40 mm Hg) is more than twice the magnitude necessary for the normal rate of filtration. In other words, GFR should be twice as great as is measured. The glomerular-pressure studies of Navar (1970) confirm the molecular studies of Pappenheimer (1955).

Only micropuncture studies of glomerular capsular fluid can unequivocally show whether inulin filters freely and at the same rate as PAH through glomerular membranes and the present studies suggest that this can only occur if passage of the solutes is by bulk transport and the membrane pore diameter is 200 Å or greater.

The situation in the rat is of interest here, for Berglund and his colleagues (Berglund, 1964, 1965; Berglund, Engberg, Persson & Ulfendahl, 1969) found that GFR decreased by about 30% as the molecular weight of the indicator substance increased from 400 to 6000 (Table 4). The point at which the clearance of polyethylene glycol became restricted in the rat was at a molecular weight of 4000 (Berglund, 1968) compared with 6000 in the dog (Shaffer, Critchfield & Carpenter, 1948). Berglund (1964, 1965) concluded that inulin clearance gave an underestimate of GFR in the rat and attributed this to the small size of the glomerular pores in this species.

His results suggest that filterability of inulin in the rat is 70% while our results suggest that filterability is 40% with a pore diameter of 100 Å and 100% with one of 200 Å. This indicates a pore size of about 150 Å in the rat; presumably, in other mammals glomerular pore size is greater than this though Pitts (1951) estimates it to be 75–100 Å in man while Verniory, Du Bois, Decoodt, Gassee and Lambert (1973) estimate it to be 100 Å in the dog.

If the glomerular pores in mammals are too small to allow free filtration of inulin then most of the measurements of tubular function based on inulin

clearance will need reconsideration: tubular secretion rates would be overestimated or reabsorption could even be mistaken for secretion.

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