DIFFUSION OF PROTEIN MOLECULES THROUGH MEMBRANES OF CONTROLLED PORE SIZE[1]

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Membrane filters which supposedly have definite and nearly uniform pore sizes have recently become available commercially. The diffusion of some proteins through such membranes has been measured in a simple apparatus. Comparison of the diffusion rates for proteins of increasing molecular weight, ranging from lysozyme to urease, shows that membranes of appropriate pore size can sift protein molecules, presumably in accordance with their molecular dimensions; the separation is much sharper than that attainable by free diffusion. Thus it appears that information concerning the size and shape of protein molecules may be obtained in this simple way.

The apparatus employed is represented in Fig. 1. When assembled, the diffusion cell consists of two compartments of equal volume, separated by a filter membrane sandwiched between two plates. A window cut in the plates exposes a definite area of the membrane to the solution. Equal volumes (usually 8 ml.) of 0.02M phosphate buffer and of 0.1-1% protein solution in the buffer were put in compartments A and B, respectively, as nearly at the same time as possible. The contents of both compartments were stirred. At appropriate intervals of time, small (ca. 0.1 ml.) aliquot portions were withdrawn from each compartment, and the concentration of protein was determined spectrophotometrically. Experiments were done with three types of filter membrane, with pore sizes of 10, 50 and 450-mμ diameter, purchased from the Millipore Filter Corp., New Bedford, Massachusetts.

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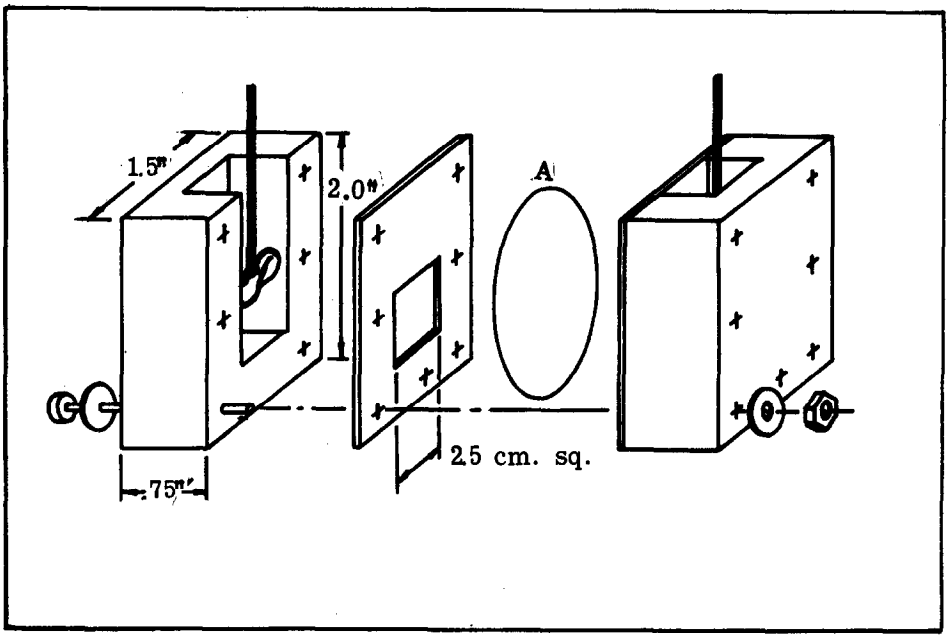

Fig. 1. Partially exploded diagram of apparatus. Cell is made of lucite; A is 47-mm. filter membrane.

Diffusion cells similar in principle though different in details of construction have been used by other investigators and discussed by Gosting (1956). In these cells, which have a thick sintered-glass or alundum diaphragm, it is first necessary to establish a state of steady-state diffusion in the diaphragm, after which the concentrations of diffusible substance in A and B are given by equation (1) (Gosting, 1956),

$$D = \frac{1}{\beta t} \ln \left(\frac{C_B^0 - C_A^0}{C_B - C_A} \right) \qquad (1)$$

where t is the time elapsed from the chosen starting time, C^0 is the initial concentration at that time, D is the diffusion coefficient, and β is a cell constant. This constant is best determined empirically with a substance of accurately known D, but can be related to the characteristics of the cell and

of an idealized diaphragm. For a diaphragm consisting of thin straight tubes of total crossection a and length h, β is given by equation (2) ($V_A = V_B = V$) (Gosting, 1956):

$$\beta = \frac{2a}{hV} \tag{2}$$

Owing to the thinness of the "Millipore" membrane, 0.0150 cm., the amount of solution and the time necessary to attain steady-state diffusion can be neglected, and the time interval can be determined from the time the cell is filled. Equation (1) then reduces to (3):

$$\ln \frac{C_B^o - 2C_A}{C_B^o} = -\beta D t \tag{3}$$

For low values of the fraction diffused, $F_A = C_A/C_B^o$, the approximation $\ln (1 + x) \cong x$ holds within 10%, and one obtains expression (4):

$$F_A = \frac{C_A}{C_B^o} = \frac{1}{2} \beta D t \tag{4}$$

Thus the initial portion of the plot of F_A versus t should be linear and its slope proportional to D. By the same token, D should be inversely proportional to the time required for, say, 10% diffusion:

$$T_{0.1} = \frac{0.2}{\beta D} \tag{5}$$

Fig. 2 shows representative data obtained with 450-mμ membranes, and it is seen that the results are in qualitative agreement with equation (3).

Table I gives some numerical data. The values of $T_{0.1}$ were determined in duplicate or triplicate experiments, each with a fresh filter membrane,

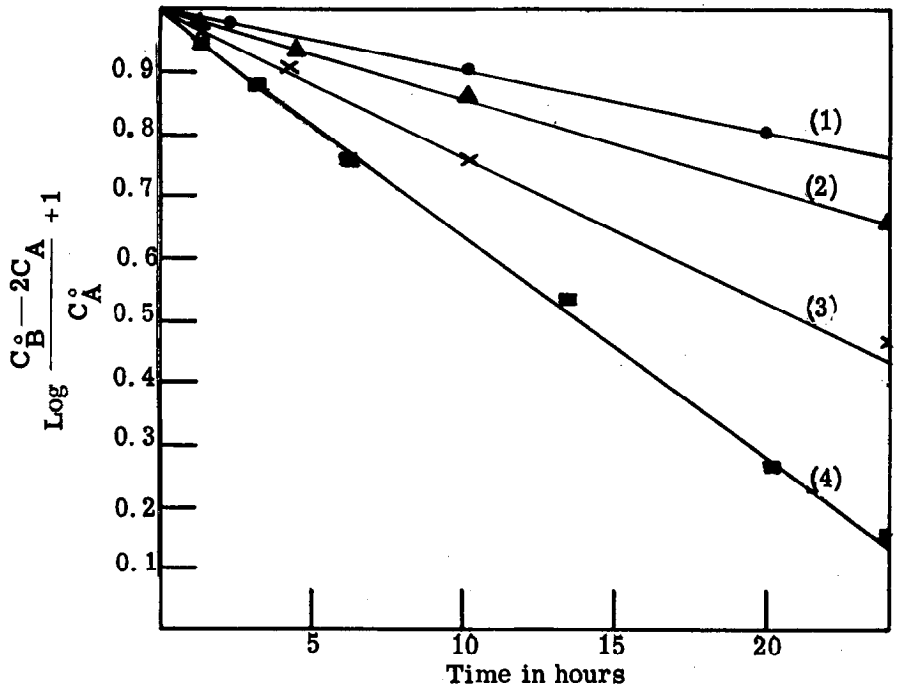

Fig. 2. Diffusion through 450-mμ membranes: (1) urease; (2) serum albumin; (3) ovalbumin; (4) lysozyme.

TABLE I

VALUES OF $T_{0.1}$ WITH VARIOUS FILTERS

Substance	M. W.	D 10^{-7} cm^2 sec^{-1}	$T_{0.1}$ in hours		
			450-mμ filter	50-mμ filter	10-mμ filter
Lysozyme	14,000	10.0	2.5	3.5	4.4
Ovalbumin	45,000	7.3	3.9	5.5	7.9
Serum albumin	69,000	6.8	6.6	18	∞*
Urease	480,000	3.4	10.2	∞*	∞*

* < 1% in 24 hours

and found to agree within 10%. This indicates the characteristics of the filters are fairly reproducible; no attempt was made, in these preliminary ex-

periments, to determine with high precision the repeatibility of β values (all filters were from one lot). The values of D listed in the Table are taken from the literature (Edsall, 1952; Gosting, 1956); while the values may not be exactly appropriate for the conditions used, their accuracy should suffice for the present purpose.

Equation (3) predicts that the product $T_{0.1}D$ should be constant for membranes of the same type. Actually, the experimental data obtained with 450-mμ membranes give values ranging from 2.5 to 4.5 (X 10^{-6} X 1/3600 cm^{-2}), not in good quantitative agreement with the prediction. However, the agreement is sufficiently good to indicate that free diffusion of the protein molecules takes place through these membranes in a nearly "normal" manner. From the values of β one can calculate $a \sim 0.4$ cm^2 (equation (2); h = 0.015 cm., V = 8 cm^3); this is a rather small fraction of the surface exposed to diffusion (6.25 cm^2) but not an unreasonable value.

The important results to be noted are those obtained with the membranes of smaller pore size. For a small protein molecule, like lysozyme, $T_{0.1}$ increases by a small amount, which might reasonably be ascribed to a decrease in a, the effective crossectional area available for free diffusion, in the denser filters. But a larger protein, like serum albumin, appears to be "sifted out" by the 10-mμ membrane; only a very small amount of protein is passed in the course of 24 hours. This indicates that there are no pores in the membrane large enough to pass this protein, i.e. that a becomes essentially zero. A similar effect is observed with urease and the 50-mμ membranes.

It is especially interesting to note the difference between two proteins of nearly the same molecular weight, ovalbumin and serum albumin. The values of D for these proteins are quite close, and, if allowed to diffuse freely, they would do so at nearly the same speed; however, the 10-mμ "Millipore" mem-

brane separates them sharply. It has been suggested (Low, 1952), though it is by no means certain (Loeb and Scheraga, 1956; Tanford, 1957) that the serum albumin molecule might be a prolate ellipsoid with a major axis 150 Å long, and it is easy to visualize how such a molecule would be prevented from passing the membrane. It would be naive to believe that sifting of molecules on a geometric basis is the only effect involved; doubtless, specific interactions between the molecules and the membrane material play a part in the diffusion process. Experiments are currently in progress to clarify these matters; it is planned, inter alia, to measure the diffusion of polystyrene latices of known particle size, by means of which it might be possible to "calibrate" the membranes empirically with respect to their "mesh size."

The results obtained with urease clearly indicate it to be a large molecule, in accordance with the molecular weight reported (Sumner et al., 1938; Creeth and Nichol, 1960). Several years ago, Hand (1939) reported some diffusion experiments, on the basis of which he suggested the existence of an enzymatically active subunit of molecular weight 17,000. The present results are not in accord with that report; particles of this molecular weight would diffuse readily through 50- and even 10-mμ membranes, contrary to what has been observed. This result illustrates how diffusion experiments may be utilized to advantage for purposes other than the direct determination of molecular size and shape.

The work described bears a close relationship to that recently done by Craig and co-workers with dialysis tubing (Goldstein and Craig, 1960; Craig, 1960). We believe the apparatus used in this work had some advantages; it is easily operated in a precisely controlled manner, and the results can be straightforwardly related to the diffusion equations. The principal advantage, however, is the larger range of pore sizes available; whereas Craig et al. found it necessary to "stretch" dialysis tubing before utilizing it in conjunction with proteins above ca. 30,000 molecular weight, there is no upper

limit to the range of the filter membranes commercially available (even though not all desirable intermediate sizes may be available).

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