Diffusion of Drugs in Modified Gelatin Films

By RICHARD H. JOHNSON

The diffusion coefficients of several physiologically active compounds have been determined in crosslinked thiolated gelatin films. The use of silica diffusion cells made possible direct ultraviolet spectrophotometric determination at the various time intervals of the solute concentration. Seven model compounds representing ionic and nonionic drugs were studied, including nicotinamide, chlorphenesin carbamate, methylprednisolone, nicotinic acid, methscopolamine bromide, sodium novobiocin, and cyanocobalamin. A correlation was shown between diffusibility and molecular weight of the drugs. Extensive binding of nicotinic acid was noted.

THE STUDY OF the diffusional behavior of derived collagen films has been hampered by the high solubility of these proteinaceous substances in water. Various techniques have been applied which alter the solubility characteristics of these polymers. For example, formaldehyde (1), tannic acid (2), and chromium salts (3) have been used to crosslink various proteins. The resultant increase in molecular weight usually has been accompanied by a decrease in water solubility.

Benesch and Benesch (4) have reported another method of crosslinking proteins. They reacted a thiolactone with the free amino groups of gelatin, thereby attaching free sulfhydryl groups to the protein chain through peptide bonds. Oxidation of thiolated gelatin solutions or gels results in intermolecular crosslinking through disulfide bridges. The resulting gels are insoluble in water.

The purpose of this investigation was to study the diffusion of drugs in crosslinked thiolated gelatin films.

EXPERIMENTAL

Preparation of the Films.—A 5% solution of thiolated gelatin[1] was adjusted to a pH of 7.1–7.4 with 1 *N* sodium hydroxide. A film was cast on glass using a Gardner film casting knife[2] at gate openings of between 0.125 and 0.300 cm The plate was then stored at 5° for 5 to 7 days to allow crosslinking to occur.

Preparation of the Solutions.—The compounds to be studied were dissolved in distilled water and ultraviolet absorptivity determined. Appropriate dilutions were made, so that the solute concentrations in either cell could be determined directly in the Cary model 11 spectrophotometer.

Diffusion Apparatus.—Figure 1 is a diagrammatic drawing of the diffusion apparatus. The dimensions of each of the two cells, A and B, are 33 × 24 × 10 mm. The theoretical volume of each cell is 7.9 cm.^{3} and the effective membrane area 2.4 cm.^{2}.

Procedure.—Cell A was filled with 7 ml. of solution of the test compound. The film, which had been hydrated and the thickness determined, was placed between the two cells. The cells were then bound together by encircling the narrow sides with Polyken tape.[3]

Cell B was then filled with 7 ml. of water through a small hole in the base of the cell which was then sealed with the tape.

To provide suitable blanks for the spectrophotometric determination of the solute concentration, a duplicate apparatus was assembled. In this case both cells were filled with 7 ml. of water.

The diffusion vessels were placed horizontally with respect to the longest dimension in a thermostated shaker bath.[4] A constant temperature of 37° (± 0.02) was maintained. The reciprocating motion of the shaker (120 c.p.m.) provided continuous stirring of the liquid adjacent to the film so that diffusion in the film was rate determining (5).

Seven physiologically active compounds representing both ionic and nonionic drugs were diffused. These drugs, listed in the order of increasing molecular weights, were: nicotinamide, nicotinic acid, chlorphenesin carbamate, methylprednisolone, methscopolamine bromide, sodium novobiocin, and cyanocobalamin.

RESULTS

The data were treated in a conventional manner employing Fick's law, assuming that the concentration gradient across the membrane was linear.[5]

The data were treated according to

$$\log\left[\frac{C_a - C_b}{C_0}\right] \cdot \frac{hv}{0.869\,(S)} = -D't \quad \text{(Eq. 1)}$$

where

D' = diffusion coefficient in cm.^{2} hr.$^{-1}$,
t = time in hours,
C_a and C_b = the concentration of solute in cells A and B in mg. ml.$^{-1}$ at time t,
C_0 = initial concentration in cell A in mg. ml.$^{-1}$,
h = hydrated thickness of the film in centimeters,
S = surface area of the film in cm.^{2}, and
v = volume in ml. which is the same on both sides of the membrane.

Similar mathematical treatment of diffusion through films in closed systems has been described previously (5, 6).

This equation assumes that data from diffusion studies using films of different thicknesses can be combined and characterized by a single diffusion coefficient. Using methylprednisolone as the solute, four runs were made employing films of different

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[1] Supplied as Thiogel by the Schwarz Bioresearch Corp.
[2] Manufactured by Gardner Laboratory, Inc., Bethesda, Md.
[3] Supplied by the Kendall Co.
[4] Model 2156 supplied by Research Specialties Co.
[5] The following calculations using an equation provided by Jost (7) show that steady-state conditions should be attained in a few minutes.

$$L = \frac{h^2}{6D'} = \frac{(4.83 \times 10^{-2}\ \text{cm.})^2}{6\ (0.385 \times 10^{-2}\ \text{cm.}^2\ \text{hr.}^{-1})}$$
$$= 0.101\ \text{hr.} \simeq 6.06\ \text{min.}$$

where L = time lag, *i.e.*, time required for solute to permeate membrane, h = film thickness, and D' = diffusion coefficient of cyanocobalamin. This compound was the least diffusible of those studied. (See Table I.)

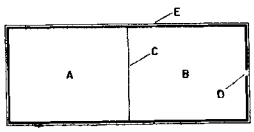

Fig. 1.—Diagrammatic drawing of diffusion apparatus. Solution of drug was introduced into cell A which was covered then with film C. The open end of cell B was placed over the film, and both cells were encircled by tape E, thereby holding them together. Water was introduced into cell B through hole D, which was then sealed with tape.

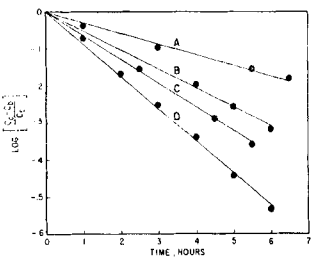

Fig. 2.—Diffusion of methylprednisolone across films of various thicknesses at 37° C. Film thickness: A, 7.87×10^{-2} cm.; B, 3.81×10^{-2} cm.; C, 3.18×10^{-2} cm.; D, 1.83×10^{-2} cm.

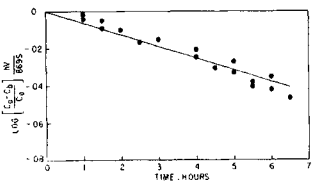

Fig. 3.—Combined data of methylprednisolone runs (Fig. 2) plotted according to Eq. 1.

TABLE I.—DIFFUSION COEFFICIENTS OF VARIOUS DRUGS IN CROSSLINKED THIOLATED GELATIN FILMS AT 37°

Drug	Mol. Wt. (Gm. mole^{-1})	Initial M Concn.	$D' \times 10^2$ (cm.^{2} hr.$^{-1}$)
Nonionic			
Nicotinamide	122.1	4.91×10^{-4}	2.168
Chlorphenesin carbamate	245.7	1.63×10^{-4}	1.475
Methylprednisolone	347.5	1.60×10^{-4}	0.641
Ionic			
Nicotinic acid	123.1	4.87×10^{-4}	0.553
Methscopolamine bromide	398.3	1.0×10^{-2}	2.286
Sodium novobiocin	634.6	9.12×10^{-5}	0.929
Cyanocobalamin	1357.4	7.37×10^{-5}	0.385

Increase ↓ Decrease ↓

thicknesses. Log $[(C_a - C_b)/C_0]$ *versus* time plots made from spectrophotometric determinations of C_a and C_b are shown in Fig. 2. Four different slopes were obtained corresponding to the thickness of the film used. The data then were combined and plotted according to Eq. 1 (Fig. 3). The linear relationship of diffusion rate and film thickness implied by Eq. 1 is evidenced by the fit of the least-squares regression line to the data.

A comparison between the diffusion coefficients and molecular weights is shown in Table I. Also listed are the initial molar concentrations employed. An examination of this table and the plots in Figs. 4 and 5 indicate a relationship between molecular weight and diffusivity, particularly in the case of the nonionic substances.

The following equation, which deals with the diffusivity of certain compounds having molecular weights from 60 to 500, was reported first by Thovert (8) and later employed by Ballard and Nelson (9) in the study of the absorption of drugs from subcutaneous implants:

$$DM^{1/2} = k$$

where M is the molecular weight, and k is a constant.

Figure 6 is a plot of the diffusion coefficients of the seven drugs *versus* the reciprocal of the square root of their molecular weights. This plot suggests an inverse relationship of diffusivity to the molecular size of the following five compounds: nicotinamide, chlorphenesin carbamate, sodium novobiocin, methylprednisolone, and cyanocobalamin. Nicotinic acid diffused slower than one would predict

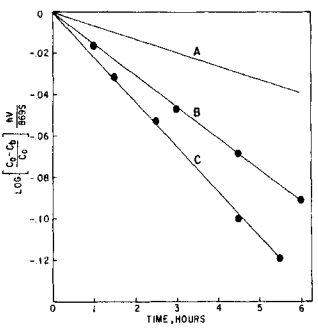

Fig. 4.—Diffusion plots of the nonionic drugs at 37° C. Key: A, methylprednisolone using linear regression line in Fig. 3; B, chlorphenesin carbamate; C, nicotinamide.

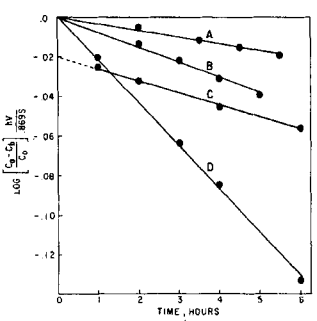

Fig. 5.—Diffusion plots of the ionic drugs at 37° C. Key: A, cyanocobalamin; B, sodium novobiocin; C, nicotinic acid; D, methscopolamine bromide.

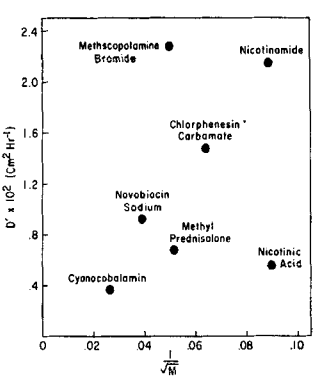

Fig. 6.—Plot showing relationship of diffusion rate to molecular weight.

by this relationship. Extensive binding of nicotinic acid to the thiolated gelatin film occurred in this study. When binding simultaneously occurs with diffusion, Eq. 1 may still be approximately valid as long as the binding occurs rapidly in relation to experimental times as in the case of nicotinic acid. With the resultant loss of diffusing solute, $C_a + C_b < C_0$, and the y intercept of the diffusion plot is negative. (See Fig. 5.) The diffusion constant for methscopolamine bromide was greater than would be predicted from its molecular weight.

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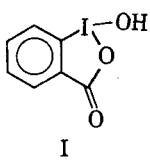
Chemistry and Biochemistry of Polyvalent Iodine Compounds III. Acute Toxicity of 1,3-Dihydro-1-hydroxy-3-oxo-1,2-benziodoxole

By WALTER WOLF, ALLEN WEINER, and ROBERT WEISBERG

The acute toxicity of 1,3-dihydro-1-hydroxy-3-oxo-1,2-benziodoxole was re-evaluated. An LD_{50} of 175 mg./Kg. was determined for mice upon intraperitoneal injection of the title compound in a buffered suspension at pH 7.48. The symptoms observed upon injection of this compound in toxic doses were incoordination, lethargy, and respiratory depression. Upon autopsy, no observable lesions could be noted.

CHINARD REPORTED (1) in 1942 that intraperitoneal injections of *o*-iodosobenzoic acid to mice resulted in symptoms of shock with death within 15 minutes. His form of administration was to dissolve the *o*-iodosobenzoic acid in an equivalent amount of potassium hydroxide in a total volume of 1.5 ml. On examination of the dead animals, he found approximately 5 ml. of a gelatinous clear exudate with scattered petechial hemorrhages on the mesenteries and intestines. A dose of 15 mg. administered subcutaneously failed to produce an acute toxic effect. He observed a gelatinous exudate, but it appeared to be reabsorbed within a short period with no observed after effects.

Recent work (2, 3) has substantiated the suggestion that *o*-iodosobenzoic acid is actually a heterocyclic compound of structure I, 1,3-dihydro-1-hydroxy-3-oxo-1,2-benziodoxole, whose pKa at 25° is 7.35. A check of the pH of the solutions, prepared as indicated by Chinard, gave values of pH 11 and above, thus allowing for the possibility that the type of toxic effects observed by this author were due to the high alkalinity of the injected solutions rather than to their content in polyvalent iodine derivatives.



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In view of the continued interest in compounds of polyvalent iodine, it was felt desirable to redetermine the true toxicity of 1,3-dihydro-1-hydroxy-3-oxo-1,2-benziodoxole.

EXPERIMENTAL

Products.—1,3 - Dihydro - 1 - hydroxy - 3 - oxo-1,2-benziodoxole was prepared by the method of Meyer and Askenasy (4), as modified by Wolf and Hsu (2, 5). The product used had an iodometric purity of at least 95%. All other products used were of commercial origin and purified when necessary.

Preparation of Suspension.—Compound I is dried well and ground in a mortar to an extremely fine powder. This finely divided powder is triturated with a few milliliters of a Sorensen phosphate buffer, pH 7.48, $^1/_{15}$ *M*, containing 0.3% methylcellulose. Once a homogenous mass is achieved, taking care that no clumps are left, the mixture is brought up to the final volume desired with the same 0.3% methylcellulose-buffer solution. A drop of *n*-octyl alcohol should be added at this moment to prevent foaming and clumping upon shaking. Several glass beads may be added to aid agitating in the suspension. Suspensions prepared in this manner were still perfectly homogeneous after shaking, 3 months after originally prepared, and the starting material had suffered no chemical changes.[1]

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[1] The authors have observed, however, that nonmethylated cellulose (paper) readily reduces the benziodoxole ring, even in the dry state. (Wolf, W., and Liberman, P., unpublished results.)