[Chem. Pharm. Bull. 24(10)2345—2349(1976)]

UDC 615.31.011.3 : 612.014.1

The Effect of Charges on Permeabilities of Drugs through Collagen Membranes[1)]

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(Received January 12, 1976)

Permeabilities of acetaminophen, bucolome (5-*n*-butyl-1-cyclohexyl-2,4,6-trioxoperhydropyrimidine), salicylic acid, aminopyrine, sulfamethizole, sulfisomidine, and *p*-aminobenzoic acid through a collagen membrane were measured at different pH values. The effect of pH of the media on permeation of acetaminophen, an unionizable drug in the pH range studied, was minor. Drugs in molecular form permeated faster than those in ionic form. Amphoteric drugs in cationic form permeated slower than those in corresponding anionic form. Little effect of charges of the drug molecules was observed on drug permeation through a cellophane membrane.

Increasing numbers of reports have dealt with the permeation of drugs through partition membranes such as silicone membranes.[3)] The permeation through partition membranes, however, depends primarily on the solubility of drugs in the membranes. Drugs with little affinity toward such membranes are not likely to permeate through them. These drugs, however, are expected to permeate through dialysis membranes.

Among dialysis membranes, cellophane has been extensively used as a hemodialysis membrane and a large volume of dialysis data is available.[4)] Permeation of drugs through cellophane has also been examined as a part of the studies on physicochemical approach to biopharmaceutical phenomena.[5)] Recently collagen has been found some use in biomedical fields because of its favorable characteristics such as good adaptability to the body, small antigenesity, digestability in the body and possibility of chemical modification to suit its need. Collagen membranes have also been evaluated as dialysis membranes.[6)] Since collagen is protein, it carries charges. The interaction of charges on collagen and those of drug molecules may modify the permeation rate of drugs.

Although the permeability of urea and other metabolic products through a collagen membrane has been studied,[7)] few reports have dealt with the permeation of drugs through collagen membranes. Besides the possible use of collagen membranes for hemodialysis, collagen was examined as to its suitability for use as a release rate-limiting barrier in the drug delivery system for the eye.[8)] It seemed worthwhile to further evaluate the possible use of collagen in the control of the drug release. As a part of such an evaluation of collagen mem-

1) This work was presented at the 95th Annual Meeting of Pharmaceutical Society of Japan, Nishinomiya, April, 1975.
2) Location: *Kita-12 Nishi-6, Kita-ku, Sapporo 060 Japan.*
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6) K.H. Stenzel, J.F. Sullivan, T. Miyata, and A.L. Rubin, *Trans. Amer. Soc. Artif. Int. Organs*, **15**, 114 (1969).
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branes, the effect of pH of the media, *i.e.*, the effect of the charge state of a collagen membrane and drugs, on the permeation of neutral, acidic, basic and amphoteric drugs through a collagen membrane was examined. This report constitutes results of these studies.

Experimental

Materials——A roll of a collagen membrane was kindly supplied by Research Laboratories of Japan Leather Company, Tokyo. The membrane had been prepared by the method previously described.[9] Briefly it involves the following steps for the preparation. Native collagen from calf skin was solubilized with proctase and purified. A mixture of solubilized collagen and collagen fibers was extruded into the coagulating bath. The membrane was then neutralized, washed and dried. It was strengthened by crosslinking with ultraviolet(UV) irradiation. The calculated pore size of the UV irradiated collagen membrane was reported to be 2.6 nm[7] which is close to the pore size of the Visking cellulose membrane (2.4 nm). The average thickness of the collagen membrane when measured with Mitsutoyo micrometer, Type 107-101A was about 0.039 mm in dry state and about 0.063 mm after hydration. The average thickness of the wet Visking cellulose membrane (18/32 type, Union Carbide Corp., Chicago) was 0.055 mm.

Drugs except for bucolome were of pharmacopeial grade. *p*-Aminobenzoic acid was of reagent grade from Wako Pure Chemical Industries. Buffering agents were of reagent grade and 0.1 M citric acid and 0.2 M disodium hydrogen phosphate were mixed in various proportions to prepare buffer solutions of different pH's. Hydrochloric acid (0.1 N) was used to obtain a solution of pH 1.1.

The Dialysis Cell——Glass dialysis cells were similar to those of Nozawa and others.[10] They are composed of a pair of cylindrical half cells with an O-ring and the membrane in-between. The capacity of each half cell was 20 ml and the surface area of the membrane was 5.3 cm^2. Vertical tubes in both half cells served as filling and sampling ports.

Permeation Procedure——A piece of the collagen membrane which had been immersed in water to be hydrated was clamped between the two half cells and the assembled cell was placed on a platform in the water bath maintained at 37°. A buffered drug solution was placed in the donor compartment and the blank buffer solution in the receptor compartment. Both solutions had been warmed to 37° before pipettied into each compartment. The cell was shaken horizontally at a rate of 75 cpm.

At suitable time intervals, small volume (0.1—1.5 ml) samples were removed from both cell compartments, diluted with a proper buffer solution to adjust pH and assayed spectrophotometrically for the drug at the wavelength of the maximum absorbance.

Time course of the drug permeation was plotted according to the following relationship,[11]

$$\ln \frac{C_d - C_r}{C_o} = -kt$$

where C_d and C_r are concentrations of a drug in the donor and receptor compartments respectively at time t C_o the initial concentration of the drug in the donor compartment, and k the apparent permeability.

Results

Effect of pH on the Membrane Permeability of Uncharged Drugs

Acidic and basic groups in collagen molecules ionize depending upon the pH of the media. This change in charge due to ionization may modify the membrane permeability of drugs. In order to examine the effect of changes in the charge state of the membrane itself on the permeation of drugs, the permeability of a drug which remains unionized over the physiological pH range was studied. Acetaminophen (pK_a=10)[12] was selected for this purpose. The permeation behavior of acetaminophen over pH 1.1—8.8 was examined at 5 pH values (1.1, 2.0, 3.0, 5.2, 7.1, and 8.8). No relation was found between pH and the membrane permeability of acetaminophen over this pH range. Thus it may be concluded that the effect of the pH of the medium on the membrane permeability of neutral drugs is small.

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10) Y. Nozawa, N. Yano, and F. Higashide, *Yakuzaigaku*, **33**, 119 (1973).
11) H.B. Kostenbauder, H.G. Boxenbaum, and P.P. DeLuca, *J. Pharm. Sci.*, **58**, 753 (1969).
12) Y.P. Chow and A.J. Repta, *J. Pharm. Sci.*, **61**, 1454 (1972).

Effect of pH on the Membrane Permeability of Acidic Drugs

As a representative of acidic drugs, an antiinflammatory drug bucolome, 5-*n*-butyl-1-cyclohexyl-2,4,6-trioxoperhydropyrimidine (pK_a=4.4),[13] was examined. At pH 2.4 where the drug is in its molecular form, it permeated faster than at pH 6.5 where it is in anionic form (Fig. 1). The difference in permeability between the molecular and anionic forms was, however, rather small. Such a tendency was observed with salicylic acid also.

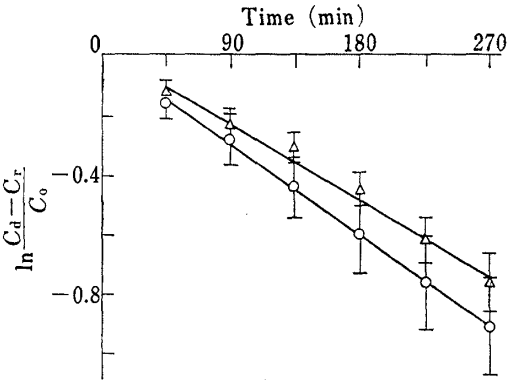

Fig. 1. Permeation of Bucolome (0.3 mM Initial Concn.) through a Collagen Membrane at 37° at pH 2.4 (○) and at pH 6.5 (△)

means of 5 measurements with standard deviations

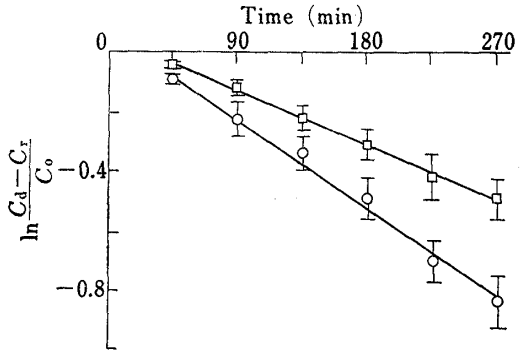

Fig. 2. Permeation of Aminopyrine (1.0 mM Initial Concn.) through a Collagen Membrane at 37° at pH 3.1 (□) and at pH 7.1 (○)

means of 5 measurements with standard deviations

Effect of pH on the Membrane Permeability of Basic Drugs

Aminopyrine (pK_a=5.1[14]) was used as a model basic drug. At pH 3.1 where it is in cationic form, it permeated much slower than at pH 7.1 where it is in molecular form (Fig. 2). Difference in permeability between molecular and ionic forms was greater in aminopyrine than in bucolome.

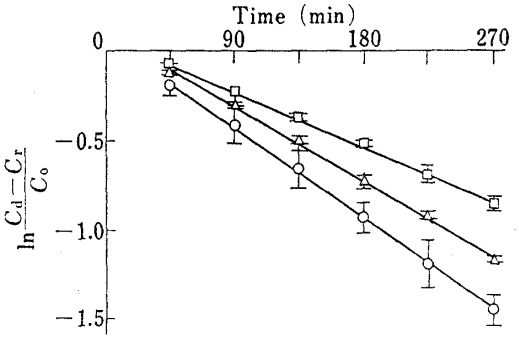

Fig. 3. Permeation of Sulfamethizole (1.0 mM Initial Concn.) through a Collagen Membrane at 37° at pH 1.4 (□), pH 3.8 (○), and pH 6.5 (△)

means of 3 measurements with standard deviations

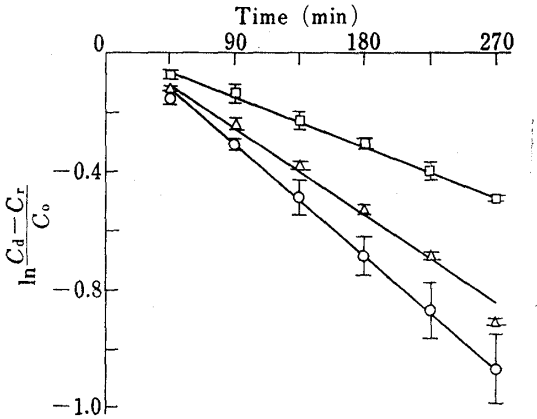

Fig. 4. Permeation of Sulfisomidine (1,0 mM Initial Concn.) through a Collagen Membrane at 37° at pH 2.1 (□), pH 5.0 (○), and pH 8.0 (△)

means of 3 measurements with standard deviations

13) H. Mima, Y. Asahi, K. Terada, T. Matsuzaki, E. Mizuta, and H. Izumi, *Ann. Rept. Takeda Res. Lab.*, **24**, 1 (1965).

14) N.W. Blacow, Ed., "Martindale," 26th ed., Pharmaceutical Press, London, 1972, p. XX.

Effect of pH on the Membrane Permeability of Amphoteric Drugs

Sulfonamides are known to have 2 pK_a values. The drugs are mostly cationic below pK_{a1}, anionic above pK_{a2}, and molecular between pK_{a1} and pK_{a2}. Fig. 3 shows the permeation pattern of sulfamethizole ($pK_{a1}=2.2$, $pK_{a2}=5.5$[15]). The permeability was smallest at pH 1.4 where the drug is in cationic form. At pH 3.8 where the drug is primarily in molecular form, the drug permeated fastest, while at pH 6.5 where it is in anionic form the permeability fell between the permeabilities at pH 1.4 and pH 3.8.

The same trend was observed with sulfisomidine ($pK_{a1}=2.4$, $pK_{a2}=7.5$[16]). The permeability was smallest at pH 2.1 (cation), greatest at pH 5.0 (molecule), and intermediate at pH 8.0 (anion) (Fig. 4). The similar results were obtained with *p*-aminobenzoic acid ($pK_{a1}=2.4$, $pK_{a2}=4.9$[17]). Again the permeability of each species was in the following order; molecule$>$anion$>$cation.

Effect of pH on the Permeability of Drugs through a Cellophane Membrane

Since cellophane membranes are widely used as dialysis membranes, the effect of pH on the permeability of drugs through a Visking cellulose membrane was examined in order to make a comparison with that through the collagen membrane.

The permeability of aminopyrine through the cellulose membrane was found unaffected by the pH of the medium (pH 3.1 and 7.1). With sulfamethizole also, differences among the permeabilities at 3 pH values (1.4, 3.8, and 6.5) were insignificant. Thus it may be concluded that the effect of charges of the drug molecule on the permeation through cellophane is negligible.

The results are summarized in Table I.

Table I. Apparent Permeability k, of Drugs through Collagen and Cellophane Membranes at 37°

Drug (pK_a value in parentheses)	pH	Predominant species	Apparent permeability $k\times10^3$ (min^{-1})	
			Collagen	Cellophane
Bucolome (4.4)	2.4	molecule	3.4	—
	6.5	anion	2.9	—
Aminopyrine (5.1)	3.1	cation	2.1	2.8
	7.1	molecule	3.3	2.8
Sulfamethizole (2.2 and 5.5)	1.4	cation	3.4	3.1
	3.8	molecule	5.6	3.5
	6.5	anion	4.6	3.6
Sulfisomidine (2.4 and 7.5)	2.1	cation	1.9	—
	5.0	molecule	4.1	—
	8.0	anion	3.4	—

Discussion

The results of the present studies showed that the pH of the medium does not affect the permeability of unionizable drugs through the collagen membrane. Namely the permeability of uncharged molecules is independent of the charge state of the collagen membrane. However, the permeabilities of ionizable drugs are influenced by the pH of the medium. In both acidic and basic drugs (bucolome and aminopyrine), the molecular forms permeated faster than the

15) P.H. Bell and R.O. Roblin, Jr., *J. Am. Chem. Soc.*, **64**, 2905 (1942).
16) T. Koizumi, T. Arita, and K. Kakemi, *Chem. Pharm. Bull.* (Tokyo), **12**, 413 (1964).
17) J.J. Christensen, D.P. Wrathall, R.M. Izatt, and D.O. Tolman, *J. Phys. Chem.*, **71**, 3001 (1967).

ionic form. Of the ionic forms, the anionic form may be considered to permeate faster than the cationic form since the permeabilities of the molecular forms of the two drugs which are widely different in nature are comparable (3.41×0^{-3} min^{-1} for bucolome and $3.3\ 10^{-3}$ min^{-1} for aminopyrine, see Table I). In amphoteric drugs (sulfamethizole and sulfisomidine), a similar order was observed for the permeability of the drug species present depending upon the pH of the medium, *i.e.*, molecule>anion>cation.

Although the exact mechanism of the impeded passage of cationic drugs over neutral or anionic drugs through the collagen membrane is not well understood, a possible mechanism may be considered. Native collagen is thought of basic protein since the number of basic groups are more than that of free carboxylic acid groups. For instance, in calf skin collagen, the number of ionizable groups per 1000 amino acid residues is reported to be about 83 basic groups and about 72 acidic groups.[18] The basic groups in the collagen membrane are mainly attributed to the basic amino acids, *i.e.* lysine, arginine, and histidine. Since the pK_{a3}'s of lysine and arginine are greater than 10, the basic groups of these amino acid residues in collagen are positively charged in the pH range of the medium studied here. As for histidine, the basic group in the residue has a pK_a value of about 6. Thus only in acidic pH this residue provides a positively charged center. The acidic groups in aspartic and glutamic acid residues are negatively charged only in the medium the pH of which is above their respective pK_{a4} values (aspartic, 3.9; glutamic, 4.3).

In acidic media (pH 1.4—3.1) where the basic and amphoteric drugs are predominantly in cationic form, the membrane is positively charged due to the protonation of the basic groups of the amino acid residues, and the acidic groups of the amino acid residues are not ionized. The electrostatic repulsion between the positively charged drug molecule and the positively charged membrane may be considered to impede the permeation of the drugs through the membrane in the acidic pH region.

In the pH region, 6.5—8.0, where the acidic and amphoteric drugs are predominantly in anionic form, the net charge state of the collagen membrane is expected to be positive, since the number of positively charged basic sites outweighs that of negatively charged acidic sites. Thus under this condition, the permeation of drugs is expected to be at least faster than the preceding case where both the drug and the membrane are of the same charge type.

When the drugs are uncharged, the charge state of the collagen membrane were shown not to influence the permeability and the permeability may be considered to be greater than that of anionic drugs through the membrane bearing net positive charges. In the latter case the drug molecules are attracted towards the membrane but the membrane tends to withhold the drug.

A lack of effects of pH on the permeation of drugs through a cellulose membrane may be rationalized by a lack of charges in the cellulose membrane. However, the present observation contradicts the reported effect of pH on the permeation of salicylic acid and cresol through a cellophane membrane.[19] The difference may be rationalized in terms of the difference in charges in a particular cellophane membrane. Although cellophane is normally uncharged, it can be negatively charged due to possible oxidation of the hydroxymethylene group in the cellulose molecule to the carboxylic group. Thus in alkaline pH, negatively charged cellulose may retard the permeation of anions.

Acknowledgement The authors are grateful to Drs. T. Miyata and M. Nishizawa, Research Laboratories of Japan Leather Company for generous supplies of the collagen membrane. The generous gift of bucolome from Takeda Chemical Industries is also appreciated.

18) T. Miyata, *Medical Electronics and Bioengineering*, **10**, 248 (1972).
19) R. Withington and J.H. Collett, *J. Pharm. Pharmacol.*, **25**, 273 (1973).