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Physical Properties of Pyrimidine and Purine Antimetabolites. II.[1] Permeation of 5-Fluorouracil and 1-(2-Tetrahydrofuryl)-5-fluorouracil through Cellophane, Collagen, and Silicone Membranes

Masahiro Nakano, Yoshiko Arakawa, Kazuhiko Juni, and Takaichi Arita

Faculty of Pharmaceutical Sciences, Hokkaido University[2]

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5-Fluorouracil and its derivative, 1-(2-tetrahydrofuryl)-5-fluorouracil (THFFU) were studied as to their permeability through cellophane, collagen, and silicone membranes. 5-Fluorouracil permeated through cellophane and collagen membranes slightly faster than THFFU. 5-Fluorouracil, however, permeated through the silicone membrane much more slowly than THFFU. Although the solubility of THFFU in silicone oil was much smaller than that in water, propylene glycol, or macrogol 400, its permeation rates through the silicone membrane from suspensions in these media were similar. The permeation rate of 5-fluorouracil through the silicone membrane from suspension in water was slightly greater than that in macrogol 400 in spite of smaller solubility in water. The permeation rate of THFFU from aqueous solutions through the silicone membrane was accelerated by the presence of sodium chloride and sodium sulfate whereas it was decelerated by sodium iodide. Permeation studies of THFFU through the silicone membrane at various pH values revealed that only the unionized form of the drug permeated through the membrane. The effect of temperature on permeability and flux of THFFU through the silicone membrane was also studied.

The method of delivery of antineoplastic agents has been recognized to be critical in cancer chemotherapy. In order to find the proper method of delivery of antineoplastic agents, physical and biopharmaceutical properties of these agents have to be evaluated. In the previous report of this series,[1] the effects of salts and temperature on solubilities of 4 antimetabolites; 5-fluorouracil, 1-(2-tetrahydrofuryl)-5-fluorouracil (THFFU), 6-mercaptopurine, and thioinosine have been presented.

A possible use of synthetic membranes as rate-determining barriers of drug release has been proposed.[3] The controlled release of nitrosoureas through silicone membranes has been reported[4,5] in an effort to improve the effectiveness and reduce the incidence of side effects of antineoplastic agents in long-term cancer chemotherapy. Studies of diffusion of THFFU through membranes from hydrophilic methacrylate gels have been published recently.[6]

In the present study, permeation behaviors of 5-fluorouracil and its tetrahydrofuryl derivative, THFFU through cellophane, collagen, and silicone membranes have been examined. In addition, effects of solvents, salts, pH and temperature on permeability of THFFU through the silicone membrane have been evaluated.

1) Part I: Y. Arakawa, M. Nakano, K. Juni, and T. Arita, *Chem. Pharm. Bull.* (Tokyo), **24**, 1654 (1976).
2) Location: *Kita-12, Nishi-6, Kita-ku, Sapporo 060, Japan.*
3) A.C. Tanquary and R.E. Lacey, Eds., "Controlled Release of Biologically Active Agents," Plenum Press, New York, 1974.
4) V. Schmidt, W. Zapol, W. Prensky, T. Wonders, I. Wodinsky, and R. Kitz, *Trans. Amer. Soc. Artif. Int. Organs*, **18**, 45 (1972).
5) M.L. Rosenblum, D.L. Bowie, and M.D. Walker, *Cancer Research*, **33**, 906 (1973).
6) J. Drobnik, P. Spacek, and O. Wichterle, *J. Biomed. Mater. Res.*, **8**, 45 (1974).

Experimental

Materials——A cellophane membrane (Visking, Type 18/32, Union Carbide Corp.) in a wet thickness of 52.0±1.7 (mean±s.d., $n=8$) μm, a UV-irradiated collagen membrane (Japan Leather Co.)[7] in a wet thickness of 29.3±2.4 μm, and the medical grade dimethylpolysiloxane sheeting, (Silastic, non-reinforced, Lot HH 0842, Dow Corning) in a labeled thickness of 5 mil (127 μm, measured thickness; 130±0.4 μm) were used. The film thicknesses were measured with Mitsutoyo Micrometer, Type 107—101A.

5-Fluorouracil and 1-(2-tetrahydrofuryl)-5-fluorouracil (mp 167°) were generously supplied from Kyowa Hakko Kogyo Co. and Taiho Pharmaceutical Co., respectively. Silicone oil (360 Medical Fluid, 20 cs, Dow Corning) was obtained through Fuji Kobunshi Co. Propylene glycol, macrogol (polyethylene glycol) 400, sodium sulfate (anhydrous), sodium chloride, sodium iodide, sodium dihydrogen phosphate (dihydrate), disodium hydrogen phosphate (12-hydrate), and trisodium phosphate (12-hydrate) were all of the purest grade available (Wako Pure Chemical Industry) and used without further purification.

Test Solutions——A suspension was prepared in a water-jacketed-beaker maintained at the temperature of the permeation studies by stirring, overnight on a magnetic stirrer, an excess amount of the drug in a solvent.

Solutions containing various salts were prepared by diluting 100 mM THFFU 2-fold by the addition of a concentrated salt stock solution and water to obtain final concentrations of 50 mM, 0.5 M, 1 M, 1 M with respect to the drug, sulfate, chloride, and iodide, respectively. When sodium iodide solution was prepared, care has been taken to ensure that pH of the salt solution falls less than 6.

Solutions at various pH values were prepared by titrating the aqueous solution of 150 mM THFFU either with sodium dihydrogen phosphate or with trisodium phosphate solution to the desired pH and adding the phosphate buffer of the corresponding pH and water to obtain the final concentration of 100 mM with respect to the drug and about 20 mM with respect to the phosphate ion. The final pH values of the resultant solutions were then measured.

Permeation Studies——For nonsteady state permeation of 5-fluorouracil and THFFU through cellophane and collagen membranes, the similar procedures to these reported earlier[7] were employed except that U-shaped diffusion cells made of a pair of L-shaped glass tubes with greaseless joints (Kokura Glass Industry Co.) were used. The membrane was placed with a Teflon O-ring between 2 halves of the cell and secured by screw clamps. The area available for diffusion was 3.14 cm^2. The cell was initially equilibrated in a thermostatted waterbath (Taiyo Incubator M^{1N}) with 20 ml of water in both arms. Water was then removed by suction and 15 ml each of a drug solution and distilled water, which had been prewarmed, was placed in each compartment. Caps were placed over each opening and the horizontal shaking(94 strokes per min.) was started. At 0.5, 1, 2, and 3 hr, 0.5 ml portions of donor and receptor solutions were pipeted and absorbance of a diluted solution was then measured at 266 nm for 5-fluorouracil and at 270 nm for THFFU with Hitachi Perkin-Elmer Spectrophotometer Model 139. The results are plotted according to the equation of Garrett and Chemburkar:[8]

$$\log \frac{C_o}{C_d - C_r} = \frac{0.869}{V_r L} P_a A t \qquad (1)$$

where C_o=initial concentration of a drug in the donor compartment, C_d and C_r=concentrations of the drug in donor compartment and receptor compartment, respectively at time t, P_a=apparent permeability, A=area available for permeation, L=membrane thickness, and V_r=volume of the receptor solution.

For steady state permeation of both drugs through the silicone membrane, similar U-shaped diffusion cells to those described above but with the available area of 1.77 cm^2 were used. The cell was initially equilibrated in a thermostatted waterbath (Haake Thermostate, Model FS) with 10 ml of distilled water in both arms. Water was then removed by suction and 5 ml of a buffer at pH 10 (0.1 M Na_2HPO_4 with a small amount of 1 N NaOH) was added to one arm to maintain a sink condition with respect to the permeable species in the receptor solution since the permeated drug completely ionizes under this condition. An equal volume of a test solution was pipetted into another arm. Caps were placed over both openings of the cell. All solutions had been equilibrated to the temperature of the permeation study before they were placed into the cell compartments. The total volume of the receptor solution was removed every day for 5-fluorouracil and every 2 hr for THFFU and replaced by 5 ml of a fresh buffer at pH 10. Absorbance of the undiluted sample was measured at 266 nm for 5-fluorouracil and at 270 nm for THFFU. Accumulated amounts of the drug permeated into the receptor solution expressed in moles were plotted against time to estimate steady-state flux.

Determination of Solubility——An excess amount of the drug was placed into a solvent in a water-jacketed beaker and equilibrated at constant temperature overnight by stirring with a magnetic bar. A portion of the equilibrated mixture was filtered quickly through a sintered glass disk and the filtrate was assayed for the drug spectrophotometrically after appropriate dilution.

7) M. Nakano, A. Kuchiki, and T. Arita, *Chem. Pharm. Bull.* (Tokyo), **24**, 2345 (1976).
8) E.R. Garrett and P.B. Chemburkar, *J. Pharm. Sci.*, **57**, 949 (1968).

Results and Discussion

Permeability through Cellophane and Collagen Membranes

Permeation behaviors of both drugs through dialysis membranes (cellophane and collagen membranes) are shown in Fig. 1 and 2. Possibly because of the presence of a tetrahydrofuryl group in THFFU, the permeability of THFFU was slightly smaller than that of 5-fluorouracil. The average permeability through the cellophane membrane was 3.6×10^{-7} cm²/sec for 5-fluorouracil and 2.8×10^{-7} cm²/sec for THFFU. The average permeability through the collagen membrane was 3.0×10^{-7} cm²/sec for 5-fluorouracil and 2.5×10^{-7} cm²/sec for THFFU.

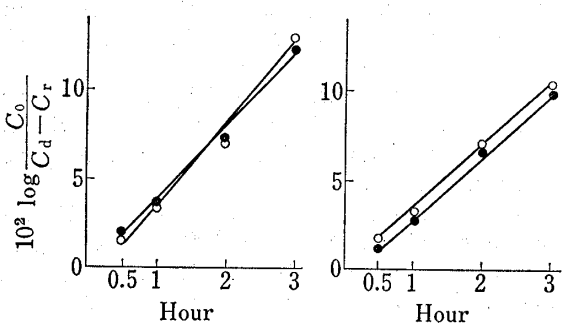

Fig. 1. Permeation of 5-Fluorouracil (Left Figure) and THFFU (Right Figure) through Cellophane Membrane at 30°

Open (○) and closed (●) circles represent two separate experiments.

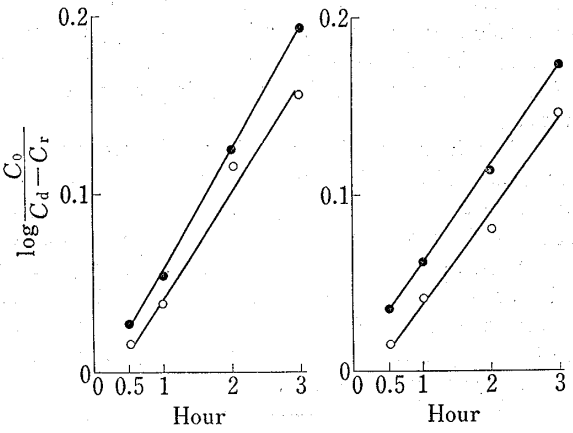

Fig. 2. Permeation of 5-Fluorouracil (Left Figure) and THFFU (Right Figure) through the Collagen Membrane at 30°

Open (○) and closed (●) circles represent two separate experiments.

Permeability through the Silicone Membranes

Permeation behaviors of both drugs from their suspensions in water and macrogol 400 through the silicone membrane are shown in Fig. 3 and 4. Permeability of 5-fluorouracil

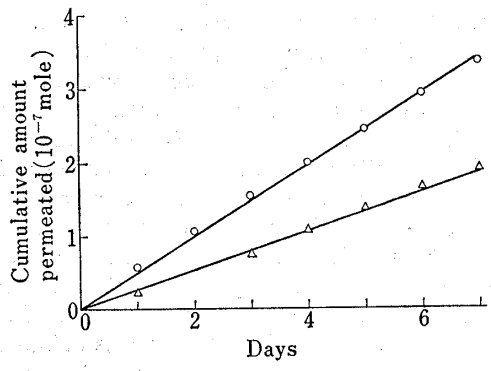

Fig. 3. Permeation of 5-Fluorouracil through Silicone Membrane from Suspensions in Water (○) and Macrogol 400 (△) at 37°

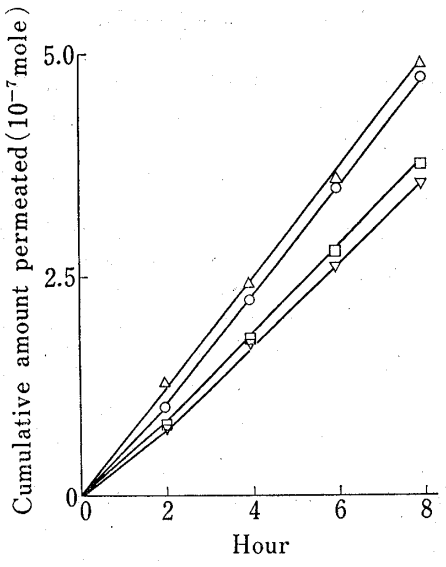

Fig. 4. Permeation of THFFU through Silicone Membrane from Suspensions in Silicone Oil (△), Water (○) Propylene Glycol (□), and Macrogol 400 (▽) at 37°

(Fig. 3, $P=0.24\times10^{-10}$ cm²/sec from aqueous suspension and 0.069×10^{-10} cm²/sec from suspension in macrogol) was much smaller than that of THFFU (Fig. 4, $P=5.2\times10^{-10}$ cm²/sec from aqueous suspension and $.5\times10^{-10}$ cm²/sec from suspension in macrogol). This observation indicates that the permeation through the partition membrane is governed primarily by partition of the drugs into the membrane material. Enhanced lipophilicity of THFFU over 5-fluorouracil is evident from solubility values of THFFU in nonpolar solvents[9] which are much greater than those of 5-fluorouracil.[10]

Effect of Solvents on Permeability

The permeation profiles of THFFU from suspensions in four media are shown in Fig. 4. In spite of more than 1000-fold difference among the solubilities in these media (Table I),

Table I. Solubility Values of THFFU in 4 Solvents at 37°

Solvent	Solubility (M)
Silicone oil	2.3×10^{-4}
Water	1.4×10^{-1}
Propylene glycol	2.1×10^{-1}
Macrogol 400	2.9×10^{-1}

the difference in permeation rate was comparatively small. Thus permeation of THFFU through the silicone rubber membrane may be considered to be membrane-controlled rather than diffusion layer-controlled.[11]

When the sink condition is maintained in the receptor solution, the flux J is expressed by the following equation[12]:

$$J=\frac{DC_m}{L} \tag{2}$$

where D is the diffusivity of the drug in the membrane, C_m the concentration of the drug in the membrane adjacent to the donor solution, and L the membrane thickness. Terms D and L may be considered to be constant for the same drug and membrane. In the presence of solid drug in the donor solution, C_m may also be considered to be constant since drug in the membrane is in equilibrium with that in the donor solution, and drug in the donor solution in turn is in equilibrium with the solid phase. The concentration of drug in the donor solution C is related to C_m by the following equation[12]:

$$C_m=KC \tag{3}$$

where K is the partition coefficient of the drug between the membrane and the solution.

Then Eq. 2 becomes:

$$J=\frac{DKC}{L} \tag{4}$$

In spite of the fact that the concentration of the saturated solution of the drug in silicone oil was only 1/600 of that in water (Table I), the flux was essentially constant (Fig. 4). This observation indicates that the partition constant between the membrane and silicone oil can be roughly 600 times as great as that between the membrane and water.

9) K. Yamanaka, A. Akazawa, and M. Yonemoto, Physicalchemical Properties of N_1-(2-Tetrahydrofuryl)-5-fluorouracil, Taiho Pharmaceutical Co., Tokyo.
10) J.J. Windheuser, J.L. Sutter, and E. Auen, *J. Pharm. Sci.*, **61**, 301 (1972).
11) S.H. Yalkowsky and G.L. Flynn, *J. Pharm. Sci.*, **63**, 1276 (1974).
12) R.W. Baker and H.K. Lonsdale in "Controlled Release of Biologically Active Agents," A.C. Tanquary and R.E. Lacey, Eds., Plenum Press, New York, 1974 pp. 15—71.

The same trend was observed with 5-fluorouracil (Fig. 3). Although the solubility of 5-fluorouracil in macrogol (3.3×10^{-1}M at 37°) was more than twice as great as that in water (1.4×10^{-1}M at 37°), the flux was smaller from the suspension in macrogol than that in water.

Similar results have been reported by Yalkowsy and Flynn[11] for the steady state flux of *p*-aminoacetophenone through silicone membrane from its saturated solution in water and propylene glycol. Although the solubility of *p*-aminoacetophenone in propylene glycol was about 18-times greater than that in water, the steady state fluxes from the saturated solutions in water and propylene glycol were almost identical.

Effect of Salts on Permeability of THFFU

The effects of sodium chloride, sodium sulfate and sodium iodide on the permeation rate of THFFU are shown in Fig. 5. Since the amount of the drug permeated was small, the concentration in the donor remained nearly constant. The plots for steady-state permeation were therefore used for the comparison of salt effects. The permeation was faster from 0.5M sodium sulfate solution and 1M sodium chloride solution than from a salt-free solution, but 1M sodium iodide solution impeded the permeation.

The effects of salts on the solubility of THFFU in aqueous solutions have been recently studied.[1] Solubility of THFFU was smaller in 1M sodium chloride solution and still smaller in 0.5M sodium sulfate solution than that in a salt-free solution whereas THFFU solubility was greater in 1M sodium iodide solution than that in pure water. Thus both sodium chloride and sulfate have a salting-out effect while sodium iodide has a salting-in effect.

Since the observed salt effect results from changes in activity coefficients, the salts are also expected to modify the apparent partition coefficient of drug between the membrane and the aqueous solution according to the following equation[13]:

$$K = \frac{C_m}{C} = \frac{\gamma}{\gamma_m} \tag{5}$$

where γ and γ_m are activity coefficients of the drug in the donor solution and that in the membrane, respectively. Whereas γ varies with species and concentration of the salt, γ_m may be considered to be constant irrespective of salt species. The relationship between J and γ or a, activity of the drug in the donor solution, can be derived from Eq. 4 and 5:

$$J = \frac{D\gamma C}{\gamma_m L} = \frac{Da}{\gamma_m L} \tag{6}$$

Equation 6 was derived by T. Higuchi[14] in his discussion on absorption from creams and ointments and later applied by Poulsen, *et al.*[15] to the study of the effect of vehicle composition on drug release.

When a salt with a salting-out tendency is added to a drug solution, the activity coefficient of the drug becomes greater than that in a salt-free solution, and consequently the flux is increased. A salt with a salting-in tendency, on the other hand, gives the activity coefficient smaller than that in a salt-free solution and decelerates the flux. The accerelated permeation of THFFU by sodium chloride and sodium sulfate and the decelerated permeation by sodium iodide can be rationalized on the basis discussed above. The present study demonstrated that the permeation rate of drugs in undersaturated solutions through the partition membrane can either be accelerated or decelerated depending upon the choice of the salt species to be added.

When the drug suspended in a salt solution (suspension) is placed in the donor compartment, on the other hand, the flux is expected to be constant irrespective of species and concent-

13) F.A. Long and W.F. McDevit, *Chem. Rev.*, **51**, 119 (1952).
14) T. Higuchi, *J. Soc. Cosmet. Chem.*, **11**, 85 (1960).
15) B.J. Poulsen, E. Young, V. Coquilla, and M. Katz, *J. Pharm. Sci.*, **57**, 928 (1968).

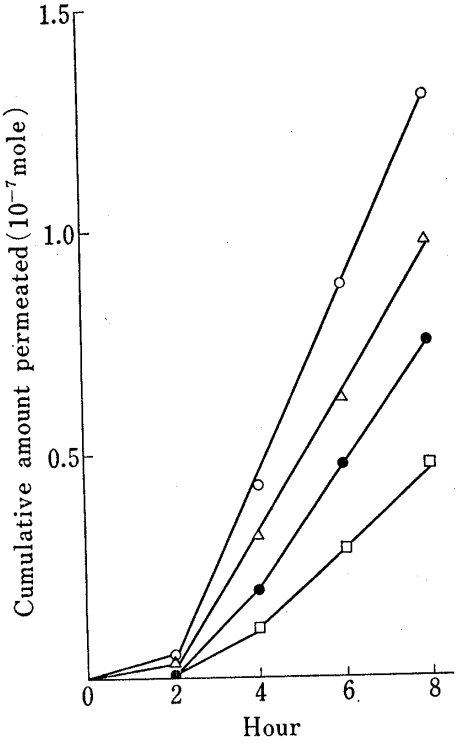

Fig. 5. Permeation of THFFU through Silicone Membrane from 50 mM Solutions in 0.5M Na_2SO_4 (○), 1M NaCl (△), Water (●), and 1M NaI (□) at 30°

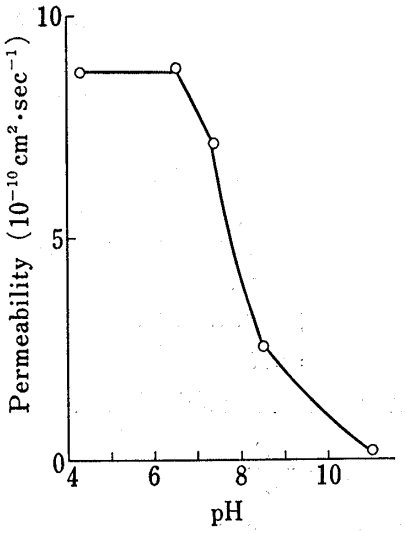

Fig. 6. Permeability Profile of THFFU through Silicone Membrane from 100 mM Solution at 37°

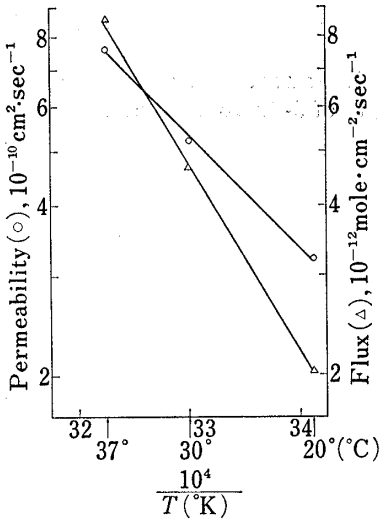

Fig. 7. Temperature Dependence of Permeability (○) and Flux (△) of THFFU through Silicone Membrane from Suspensions in Water

ration of the salt according to Eq. 6. Activity of the drug in any saturated solution which is in equiliblium with solid drug is considered to be constant at constant temperature.

Effect of pH on Permeability of THFFU

THFFU is a weak acid with a pK_a of 7.7[9] and therefore percentages of the unionized form of the drug vary with the pH of the aqueous solution. The permeability of THFFU was therefore examined in a pH range of 4.4—11.0. The results are shown in Fig. 6. In weak acid solution where the drug is predominantly in molecular form, it permeated to a significant extent. In neutral pH region the permeation rate dropped sharply as the percentage of the drug in the molecular form decreased. In alkaline pH region little permeation took place. Thus it may be concluded that only the drug in unionized form permeates through the silicone membrane as was the case with *p*-aminopropiophenone[8] and pentobarbital.[16] Thus the concentration of the permeable species C may be shown by the following equation:

$$C = fC_t \qquad (7)$$

where f is the fraction of the unionized drug and C_t the total concentration of the drug.

16) E.R. Garrett and P.B. Chemburkar, *J. Pharm. Sci.*, **57**, 1401 (1968).

Effect of Temperature on Permeability of THFFU

The dependency of the flux and permeability on temperature from saturated solutions is shown in Fig. 7. The flux was found to be more dependent on temperature than the permeability.

When a suspension of the drug is placed in the donor compartment, the flux is influenced both by the solubility of the drug in the donor solution and the diffusivity of the drug within the membrane at a particular temperature (Eq. 4). Namely, when the sink condition is maintained in the receptor solution, the flux is expressed by Eq. 4 and the permeability P by the following equation[12]:

$$P = DK \tag{8}$$

Then the relationship between the flux and permeability is represented by:

$$J = \frac{PC}{L} \tag{9}$$

Since both the solubility and diffusivity increase rapidly with the increase in temperature, the flux from saturated solution is expected to be more dependent on temperature than the permeability which increases only with the increase in diffusivity since normally the change in partition coefficient with temperature may be considered to be small.

Activation energy of permeation estimated from Fig. 7 is found to be 8.5 kcal/mole.

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