STUDIES ON DRUG ABSORPTION FROM ORAL CAVITY: PHYSICO-CHEMICAL FACTORS AFFECTING ABSORPTION FROM HAMSTER CHEEK POUCH

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A new experimental method using a hamster cheek pouch *in vivo* for studying absorption processes across the keratinized oral mucous membrane was developed and some characteristics of the oral-mucosal absorption were clarified. A positive correlation between the absorption rate and the lipophilicity was observed in 18 aromatic compounds examined. The absorption of ionizable compounds was poor in the ionized form. These results suggest that the absorption from the keratinized hamster cheek pouch is due to a passive diffusion mechanism and obeys pH-partition hypothesis. The systemic transfer of salicylic acid after intra-cheek-pouch administration could be approximated by the first-order absorption model including a lag time process and the plasma levels found were consistent with this model.

Keywords — absorption; oral mucosa; hamster cheek pouch; pharmacokinetics; physico-chemical factor; salicylic acid

INTRODUCTION

The absorption of drugs from the oral cavity has been investigated by the buccal absorption test of Beckett and Triggs¹⁾ in man. In these studies, it has been shown that the ability of a compound to cross the oral epithelia is highly dependent upon both the degree of its ionization and the lipophilicity of its unionized form.^{2–4)} The oral mucosa has been classified into three types according to function; *i.e.*, masticatory, lining and specialized mucosa. Also there are regional variations in the epithelial thickness and the degree of keratinization.⁵⁾ Although the buccal absorption test in man can easily provide data concerning the absorption rates of drugs administered in the oral cavity, the test does not show the regional differences in the absorption rates⁶⁾ or the rate-limiting step(s) (permeation barrier) of the absorption process.^{7,8)} Studies on mucosal permeability to drugs *in vitro*, using diffusion cells, provide valuable information concerning the relationship between the rate of transfer and the physico-chemical properties of substances such as lipid solubility, molecular size, chemical composition and ability to form hydrogen bonds,^{9–11)} but no information concerning the transfer process from the oral tissue to the systemic circulation.

In this paper, we describe a new experimental method for studying absorption processes across the keratinized oral mucosa *in vivo* using a hamster cheek pouch, which consists of keratinized stratified squamous epithelium, and discuss some characteristics of drug absorption through a keratinized region of oral mucosa.

MATERIALS AND METHODS

Chemicals — All chemicals used were reagent grade commercial products.

Procedure of Absorption Experiments — Male golden hamsters (80–110 g body weight) were anesthetized with urethane (1.5 g/kg, *i.p.*) and were fastened to a platform at an angle of about 55°. The cheek pouch was cleaned by multiple rinses with saline and the lumen was wiped with cotton balls to remove excess water. A vinyl tubing (o.d. 1.2 mm, i.d. 0.8 mm, Dural Plastics, Australia) was inserted into the cheek pouch and was fixed with Aron Alpha (Toagosei Chemicals, Japan) to the corner of mouth in 40 mm depth (Fig. 1). Drugs were dissolved in appropriate isotonic buffer solution (citric acid- Na_2HPO_4 at pH 2.0 to 5.0 and NaH_2PO_4 - Na_2HPO_4 at pH 6.0 and pH 7.0). One ml of the drug solution was administered into the cheek pouch. After standing for a definite period, the luminal contents

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were withdrawn and the cheek pouch was washed with the same buffer solution. The washings were combined with the luminal contents and made up to 20 ml by addition of saline. The amount of the drug remaining was determined. The disappearance from the lumen was defined as the apparent absorption.

Determination of Salicylic Acid Remaining in Tissue of Cheek Pouch — In some absorption experiments for salicylic acid, the amount of the drug remaining in the tissue of cheek pouch was

FIG. 1. Schematic Representation of Absorption Experiment for Hamster Cheek Pouch

determined. After washing out the residual salicylic acid, the cheek pouch was immediately cut off. A half N NaOH solution was added to the tissue to make the total amount 20.0 g. The tissue in alkaline solution was then placed in a boiling water bath for 30 min. After cooling the resultant solution, some impurities were extracted with chloroform. The remaining aqueous phase was made acidic with concentrated HCl and salicylic acid was extracted with chloroform. Then, salicylic acid, in the organic phase, was re-extracted with 0.1 N NaOH solution and the drug in the final aqueous layer was determined by a high pressure liquid chromatography (HPLC). For the calibration curve, appropriate amounts of salicylic acid were added to the tissue of cheek pouch and the same procedures were carried out.

Analytical Methods — *p*-Aminobenzoic acid and sulfisoxazole were diazotized following a regular procedure, coupled with 2-diethylaminoethyl-1-naphthylamine and extracted with isoamylalcohol after addition of 1 g of NaCl. The optical density of the organic layer was determined at 560 and 555 nm, respectively. HPLC was used to determine the other compounds remaining in both lumen and tissue of the cheek pouch. The chromatograph was 570B (Gasukuro Kogyo, Japan) equipped with a ultraviolet (UV) detector (502S, Gasukuro Kogyo).

TABLE I. HPLC Conditions for the Analysis of Test Compounds

Compound	Mobile phase ^{a)}	Wave-length (nm)
Salicylic acid	45 : 55 ^{b)}	215
Phenol	50 : 50 ^{b)}	220
Benzoic acid	40 : 60 ^{b)}	227
Acetylsalicylic acid	50 : 50 ^{b)}	220
Propionylsalicylic acid	50 : 50 ^{b)}	220
Butyrylsalicylic acid	40 : 60 ^{b)}	220
<i>m</i> -Hydroxybenzoic acid	50 : 50 ^{b)}	300
<i>p</i> -Hydroxybenzoic acid	50 : 50 ^{b)}	253
<i>o</i> -Toluic acid	30 : 70 ^{b)}	220
Anthranilic acid	70 : 30 : 3 ^{c)}	248
Acetanilide	40 : 60 ^{b)}	237
Acetaminophen	40 : 60 ^{b)}	240
Phenacetin	40 : 60 ^{b)}	243
Methylparaben	35 : 65 ^{b)}	254
Ethylparaben	30 : 70 ^{b)}	254
Propylparaben	25 : 75 ^{b)}	254

a) Values are expressed as volume to volume composition of two or three components.
 phosphoric acid : methanol.

c) H₂O : acetonitrile : acetic acid.

b) 0.025%

The column used was a reversed-phase Unisil Q C18 (4.0 × 300 mm, Gasukuro Kogyo). Mobile phases and the wave-lengths for determination are summarized in Table I. An aliquot of the sample solution was filtered through a 0.45 μm pore-size triacetylcellulose membrane (Fuji Photo Film, Japan) and an appropriate volume of the filtrate was injected into the liquid chromatograph. The concentration of the compound was calculated from the peak height using the calibration curve.

Plasma Concentration of Salicylic Acid

— Under urethane anesthesia, the carotic artery was cannulated with polyethylene tubing (o.d. 0.8 mm, i.d. 0.5 mm, Dural Plastics) and then heparin (500 unit/kg) was administered intravenously. In an *i.v.* administration study, salicylic acid was dissolved in saline to make 20 mM and was injected into a femoral vein (1 ml/kg = 20 μmol/kg). In the study of intra-cheek-pouch administration, salicylic acid dissolved in isotonic buffer solution at the concentration of 10 mM was administered into the cheek pouch in a similar manner as described in Procedure of Absorption Experiments (10 ml/kg = 100 μmol/kg). In both studies, blood samples (0.2 ml) were collected periodically from the cannula for 4 to 6 h after the administration and the plasma was separated immediately by centrifugation. After addition of acetonitrile, the plasma was centrifuged and the supernatant was filtered through a 0.45 μm pore-size filter (Nihon Millipore Kogyo, Japan). Salicylic acid concentrations in plasma were determined by HPLC equipped with a fluorescence detector RF-540 (Shimadzu, Japan) at 300 and 430 nm for excitation and emission, respectively.

Pharmacokinetic Studies — Plasma concentration-time data from *i.v.* and intra-cheek-pouch administration studies were simultaneously fitted to a two-compartment model (Fig. 6) using a nonlinear least-squares program, MULTI.¹²⁾ In this model, the parameters of lag times and absorption rate constants for two intra-cheek-pouch administration studies were independent of each other.

Determination of Lipophilic Index, $\log(k'_0)$, at pH 3.0 — The lipophilic index, $\log(k'_0)$, of each compound at pH 3.0 was determined by reversed-phase HPLC according to the method of Yamana *et al.*¹³⁾ The apparatus for HPLC was the same as described in Analytical Methods

and all compounds were detected at 254 nm. The mobile phase of methanol-buffer solution (pH 3.0) made of 12.6 mM citric acid, 6.4 mM Na₂HPO₄ and 10 mM NH₄Cl was run at a flow rate of 1.0 ml/min. Ammonium salt was added to block the active silanol sites of the column. Formamide was used as an unretained substance. When $\log(k')$, which was defined as follows;

$$\log(k') = \log[(t_R - t_0)/t_0] \quad (1)$$

where t_R and t_0 are the retention times of a retained peak and of an unretained peak, respectively, was plotted against methanol concentration (v/v %), reasonable linear relationships for all compounds tested ($r < -0.999$) were obtained. The lipophilic index, $\log(k'_0)$, was defined as a $\log(k')$ value extrapolated to 0% methanol.

RESULTS

Absorption from the Hamster Cheek Pouch

The absorption of 18 aromatic compounds at pH 3.0 from the hamster cheek pouch was examined. Table II summarizes the results of the absorption experiment. The percentage of absorption of compounds from the lumen of the cheek pouch in 1 h varied over a wide range. In the case of alkyl acid esters of salicylic acid, the absorption increased as the alkyl chain-lengths increased, *i.e.*, acetyl- < propionyl- < butyrylsalicylic acid. Similarly among parabens, the absorption increased in the order of methyl- < ethyl- < propylparaben. These results are in agreement with the buccal absorption data of *N-n*-alkylamines.³⁾ The absorption of both *m*-hydroxy- and *p*-hydroxybenzoic acid, the structural isomers of salicylic acid, were about one-tenth of salicylic acid. Likewise, for aminobenzoic acids, the *o*-isomer (anthranilic acid) was more absorbable than the *p*-isomer.

Effect of Luminal Concentration on the Absorption of Salicylic Acid

The effect of luminal concentration of salicylic acid on its absorption from the cheek pouch was examined in the concentration range of 1.0 to 10.0 mM. The percentages of the absorption in 1 h at pH 3.0 were 48.6, 49.8 and 48.2 at 1.0, 5.0 and 10.0 mM, respectively (Fig. 2), showing the linearity of salicylic acid absorption in the concentration range examined.

Time Course of Salicylic Acid Absorption

To clarify the process of the absorption of a drug after intra-cheek-pouch administration,

TABLE II. *Absorption of Model Compounds from the Hamster Cheek Pouch at pH 3.0*

Compound	Molecular weight	Conc. (mM)	% absorbed in 1 h ^{a)}
Phenol	94.1	1.0	34.6 ± 2.6 (4)
Benzoic acid	122.1	1.0	37.6 ± 1.5 (3)
Salicylic acid	138.1	1.0	48.6 ± 4.1 (4)
Acetylsalicylic acid	180.2	1.0	11.1 ± 1.6 (3)
Propionylsalicylic acid	194.2	1.0	25.7 ± 1.9 (4)
Butyrylsalicylic acid	208.2	1.0	40.8 ± 5.5 (4)
<i>m</i> -Hydroxybenzoic acid	138.1	5.0	6.3 ± 0.8 (4)
<i>p</i> -Hydroxybenzoic acid	138.1	5.0	6.5 ± 0.7 (4)
<i>o</i> -Toluic acid	136.2	1.0	50.6 ± 3.0 (4)
Anthranilic acid	137.1	1.0	14.3 ± 1.6 (4)
<i>p</i> -Aminobenzoic acid	137.1	1.0	8.6 ± 1.0 (4)
Slulfisoxazole	267.3	1.0	6.0 ± 0.7 (4)
Acetanilide	135.2	1.0	7.7 ± 1.9 (4)
Acetaminophen	151.2	1.0	0.9 ± 0.5 (4)
Phenacetin	179.2	1.0	16.0 ± 0.6 (3)
Methylparaben	154.2	1.0	30.1 ± 2.5 (7)
Ethylparaben	166.2	1.0	44.8 ± 0.8 (4)
Propylparaben	180.2	0.9	64.6 ± 3.2 (4)

a) Results are expressed as the mean ± S.E. with the number of experiments in parentheses.

salicylic acid was administered and the amounts of salicylic acid remaining in both the lumen and the tissue of the cheek pouch were periodically determined at pH 3.0 and at pH 4.0. Results are shown in Fig. 3. The semilogarithmic plots of the luminal salicylic acid (open circles) indicated that the disappearance from the lumen could be described by a biexponential process. However, the transfer to the systemic circulation (closed circles) was shown to be an apparent first-order process at both pH conditions. Accordingly, it is reasonable to consider that the systemic transfer after intra-cheek-pouch administration can be approximated by the first-order process after a lag time. The apparent transfer rate constants to the systemic circulation calculated from the slope of the closed circles and the lag times estimated by extrapolation were 0.49 h⁻¹ and 7 min, and 0.11 h⁻¹ and 25 min for pH 3.0 and pH 4.0, respectively.

Plasma Concentration of Salicylic Acid after Intra-Cheek-Pouch Administration

Plasma concentrations of salicylic acid after intra-cheek-pouch administration (100 μmol/kg) as a function of time are shown in Fig. 4. The plasma level *versus* time curves were clearly dependent on the pH of the administered solution. When salicylic acid was administered

at pH 3.0, the plasma concentration was rapidly increased to about 100 nmol/ml and then decreased thereafter. On the other hand, at pH 4.0, it took about 1.5–2 h to reach the maximal concentration of about 25 nmol/ml and this concentration was maintained for at least 5 h.

Pharmacokinetics of Salicylic Acid after Intra-Cheek-Pouch Administration

To confirm the plasma elimination kinetics of salicylic acid in hamsters, an *i.v.* administra-

FIG. 2. *Concentration Dependency of Salicylic Acid Absorption from the Hamster Cheek Pouch*
Results are expressed as the mean ± S.E. of at least four experiments.

tion was carried out. As shown in Fig. 5, the plasma concentrations of intravenously administered salicylic acid declined biexponentially. As the absorption from the cheek pouch can be approximated by the first-order process after a lag time (Fig. 3), the two-compartment model with a first-order absorption process shown in Fig. 6 seemed to be available as the pharmacokinetic model for intra-cheek-pouch administration of salicylic acid. Plasma concentration-time data after intra-cheek-pouch and *i.v.* administration of salicylic acid (Figs. 4 and 5) were simultaneously fitted to the model using a nonlinear least-squares program, MULTI,¹²⁾ and the pharmacokinetic parameters estimated are listed in Table III. The absorption rate constant, k_{ab} , and the lag time, t_0 , estimated here agreed with the apparent transfer rate constant for the systemic circulation and the lag time obtained from Fig. 3 at each pH condition, respectively.

Effect of Lipophilicity on the Absorption from the Hamster Cheek Pouch

$\log(k')$ values together with $\log(k'_0)$ values for 18 compounds at pH 3.0 are listed in Table IV. It has been shown that the absorption from the oral mucosa is related to the lipophilicity of the compound in both *in vivo*^{2-4,14)} and *in vitro*⁹⁻¹¹⁾ experiments. Fig. 7a shows the positive correlation ($r=0.764$) between $\log(k'_0)$ and the absorption rate at pH 3.0. The theory of diffusion through a single-layer homogeneous membrane predicts for small compounds that permeability is inversely proportional to the square root of the molecular weight (M_r).¹⁵⁾ When this concept was applied, Fig. 7b was obtained where the abscissa was altered to $\log(k'_0)/(M_r)^{1/2}$. A better correlation ($r=0.874$) was observed between $\log(k'_0)/(M_r)^{1/2}$ and the absorption rate, suggesting that not only the lipophilicity but also the molecular size may affect the absorption

FIG. 3. Time Course of Salicylic Acid Absorption from the Hamster Cheek Pouch

Semilogarithmic plots of salicylic acid remaining in lumen and in tissue of the cheek pouch (a) at pH 3.0 and (b) at pH 4.0: ○, salicylic acid remaining in luminal fluid; ●, sum of salicylic acid remaining in lumen and in tissue. (c) Tissue accumulation of salicylic acid: ●, pH 3.0; ○, pH 4.0. Results are expressed as the mean $\pm$ S.E. of at least three experiments.

from the keratinized oral mucosa.

Effect of pH on the Absorption from the Hamster Cheek Pouch

To determine the effect of pH on the absorption from the hamster cheek pouch, absorption

FIG. 4. Plasma Concentration of Salicylic Acid after Intra-Cheek-Pouch Administration

100 μ mol/kg of salicylic acid was administered into the cheek pouch: $\circ$, pH 3.0; $\bullet$, pH 4.0. Results are expressed as the mean $\pm$ S.E. of at least three experiments. Each line represents the curve fitted with the two-compartment model shown in Fig. 6.

FIG. 5. Plasma Concentration of Salicylic Acid after i.v. Administration in Hamster

20 μ mol/kg of salicylic acid was injected into the femoral vein. Results are expressed as the mean $\pm$ S.E. of three experiments. Line represents the curve fitted with the two-compartment model shown in Fig. 6.

experiments were carried out for salicylic acid, benzoic acid and phenacetin. Fig. 8 is the plots of absorption as a function of pH. The pH change was negligible throughout the experiment. The absorption of both salicylic acid and benzoic acid were decreased with the increase of the pH of the solution and almost nil at pH 7.0, where these compounds were completely ($>99\%$) ionized since the pK_a values were 3.0 and 4.2, respectively. On the other hand, the absorption of phenacetin, which is not ionized in the pH range examined, was not affected by the pH. These data suggest that the ionized form of the compound was poorly absorbable from the hamster cheek pouch although the inflection points of the pH-absorption curves were slightly shifted to the basic side compared with the pK_a values in both acids.

DISCUSSION

The morphology of the oral mucosa varies from region to region depending on function. It is likely that there are differences in permeability among structurally different regions of the oral mucosa, such as keratinized or non-keratinized.⁶⁾ One of the simplest methods directly measuring permeation rate through the oral mucosa *in vivo* is buccal absorption test of Beckett and Triggs.¹⁾ In this method, the uptake of a compound is es-

FIG. 6. Pharmacokinetic Model for Salicylic Acid in Hamster

D or D' , dose; t_0 , lag time; k_a , first-order absorption rate constant; k_e , first-order excretion rate constant; k_{12} and k_{21} , first-order transfer rate constants between two compartments; V_1 , volume of distribution of central compartment; V_2 , volume of distribution of peripheral compartment. Salicylic acid was administered by i.v. or intra-cheek-pouch administration.

timated from the differences in amount between the initial and the final solutions in the mouth. However, this method cannot provide information as to the relative permeability of different regions in the oral cavity, because the area where the absorption may have taken place cannot be confirmed. In addition, since the measurement was carried out in human volunteers, the test conditions should be restricted; that is, the agents which are thought to damage the biological membranes cannot be used for studying the

permeation barriers. Furthermore, the data from untrained volunteers showed wide deviation in this manner.^{1,16)} *In-vitro* methods, usually using diffusion cells, enable anatomically well-defined regions of mucosa to be studied, but cannot provide information concerning the transfer from oral tissue to systemic circulation. In this study we used a hamster cheek pouch, which has keratinized stratified squamous epithelia¹⁷⁾ similar to those of gingiva and hard palate in man and which could be well separated from the

TABLE III. Pharmacokinetic Parameters of Salicylic Acid in Hamster Based upon the Model Shown in Fig. 6

Pharmacokinetic parameter	Condition of administration	
	pH 3.0	pH 4.0
t_0 (min)	8.4	24.0
k_a (h ⁻¹)	0.529	0.103
k_{12} (h ⁻¹)		1.05
k_{21} (h ⁻¹)		0.71
k_e (h ⁻¹)		2.25
V_1 (l/kg)		0.125
V_2 (l/kg)		0.185

The parameters were estimated by the damping Gauss-Newton method. Since the analytical limit of the plasma salicylic acid was less than 1 nmol/ml, the reliability of the data appeared to be equivalent in each point. Thus, in estimation, $(C_i)^{-2}$ was adopted as the weight where C_i is the value of the i -th point. Akaike's information, AIC, in this estimation was -19.3 .

TABLE IV. Lipophilic Indexes of Model Compounds at pH 3.0

Compound	log (k')					log (k'_0)
	70% MeOH ^{a)}	60% MeOH	50% MeOH	40% MeOH	30% MeOH	
Phenol	N.D. ^{b)}	-0.367	-0.113	0.116	0.344	1.06
Benzoic acid	-0.514	-0.212	0.089	0.392	N.D.	1.60
Salicylic acid	N.D.	-0.583	-0.212	0.103	0.414	1.42
Acetylsalicylic acid	N.D.	-0.486	-0.174	0.103	0.412	1.30
Propionylsalicylic acid	-0.596	-0.253	0.092	0.450	N.D.	1.84
Butyrylsalicylic acid	-0.371	-0.004	0.377	0.770	N.D.	2.30
<i>m</i> -Hydroxybenzoic acid	N.D.	-0.678	-0.360	-0.058	0.252	1.18
<i>p</i> -Hydroxybenzoic acid	N.D.	-0.744	-0.423	-0.127	0.171	1.09
<i>o</i> -Toluic acid	-0.320	-0.001	0.316	0.647	N.D.	1.93
Anthranilic acid	N.D.	-0.405	-0.129	0.131	0.385	1.18
<i>p</i> -Aminobenzoic acid	N.D.	N.D.	-0.682	-0.388	-0.110	0.75
Sulfisoxazole	N.D.	-0.812	-0.439	-0.046	0.389	1.57
Acetanilide	N.D.	-0.340	-0.084	0.174	0.455	1.24
Acetaminophen	N.D.	N.D.	-0.824	-0.549	-0.253	0.60
Phenacetin	-0.441	-0.153	0.151	0.477	N.D.	1.69
Methylparaben	-0.522	-0.217	0.102	0.430	N.D.	1.70
Ethylparaben	-0.328	0.006	0.364	0.746	N.D.	2.17
Propylparaben	-0.118	0.258	0.658	N.D.	N.D.	2.59

a) Mobile phase. b) Not determined.

Results are expressed as the mean of duplicate experiments.

FIG. 7. Effect of Lipophilicity on the Absorption from the Hamster Cheek Pouch

(a) Correlation between the absorption and $\log(k'_0)$. (b) Correlation between the absorption and $\log(k'_0)/(M_r)^{1/2}$. Keys: 1, phenol; 2, benzoic acid; 3, salicylic acid; 4, acetylsalicylic acid; 5, propionylsalicylic acid; 6, butyrylsalicylic acid; 7, *m*-hydroxybenzoic acid; 8, *p*-hydroxybenzoic acid; 9, *o*-toluic acid; 10, anthranilic acid; 11, *p*-aminobenzoic acid; 12, sulfisoxazole; 13, acetanilide; 14, acetaminophen; 15, phenacetin; 16, methylparaben; 17, ethylparaben; 18, propylparaben.

FIG. 8. pH-Profiles of Absorption from the Hamster Cheek Pouch

●, 5 mM salicylic acid; ▲, 1 mM benzoic acid; ■, 1 mM phenacetin. Results are expressed as the mean $\pm$ S.E. of at least three experiments.

other oral mucosa during the absorption experiment to investigate (i) the process that a compound administered in solution disappeared from the lumen of the cheek pouch, (ii) the process that the compound transferred from the cheek pouch tissue into the systemic circulation and (iii) the factors affecting those processes.

It has been demonstrated at various sites that

the absorption rates of compounds absorbed by a passive diffusion mechanism correlated well with their lipophilicity. Recently, Evered *et al.* using the human buccal absorption test showed that there are carrier-mediated transport systems for nicotinic acid, nicotinamide,¹⁸⁾ thiamine¹⁹⁾ and glutathione²⁰⁾ in the human oral cavity. In the keratinized mucosa including hamster cheek pouch, however, the carrier-mediated transport system may not exist. As shown in Figs. 2 and 8, the absorption of salicylic acid was increased linearly with the dose and was pH-dependent, namely, the absorption was poor at the higher pH regions where this compound was completely ionized. In addition, there was a positive correlation between the absorption rates and the lipophilic indexes, which correlated well with the partition coefficients in 1-octanol-water,¹³⁾ with the 18 aromatic compounds tasted (Fig. 7). Consequently, it can be assumed that the absorption mechanism from the keratinized hamster cheek pouch is a passive diffusion. There are only a few studies concerning the absorption from the hamster cheek pouch.²¹⁻²³⁾ Whitford *et al.*²³⁾ reported that the absorption of fluoride through this keratinized epithelium occurred mainly by diffusion of undissociated acid, HF, and this agrees well with the present findings.

The data presented here involve two problems to be solved. One is a pH-shift shown in

Fig. 8. Since the pK_a values for salicylic acid and benzoic acid are 3.0 and 4.2, respectively, the pH-profiles of the absorption for both acids were shifted to basic side approximately 0.5–1.0 pH unit from each curve of unionized fraction. The shifts of pH-absorption curves have already been reported for some drugs in the gastrointestinal tract including the oral cavity and the contribution of physiological factors such as a higher acidic pH, *i.e.*, virtual pH at the membrane surface,^{24,25)} the buffering surface system,¹⁶⁾ or drug binding to the mucosal membrane surface^{26,27)} have been proposed. However, Anmo *et al.*²⁸⁾ using a recirculation method reported that the absorption of salicylic acid, salicylamide and gentisic acid from the rat oral mucosa agreed well with each dissociation curve and that the unionized form was preferentially absorbed compared with the ionized form. In this recirculation method, the recirculating rate of the drug solution was sufficiently rapid and the solution adjacent to the absorption surface was well stirred. Recently, Tsuji *et al.*²⁹⁾ clearly showed by *in vitro* interphase transport study using a two-phase rolling cell that the aqueous diffusion layer appeared to be the most reasonable explanation of the pH-shifts observed in penicillins by 0.8–2 pH units in rat stomach and small intestine, without application of physiological factors. Although the experimental proof must be required, it is difficult to rule out the effect of such an aqueous diffusion layer on the absorption from the hamster cheek pouch in the present experimental method. The other is a biexponential loss from the lumen of the cheek pouch observed in the time course study of salicylic acid absorption (Fig.3) Beckett and Pickup³⁰⁾ also showed the biexponential loss of some steroids from the oral cavity in man, in which the distribution phase was completed within 5 min. They used a two-compartment open model which meant a reversible membrane storage to explain the absorption. Since the distribution phase was completed within the shortest experimental period of 15 min in the present study, we could not construct an appropriate modeling under this condition. However, the mechanism of this phenomenon may also be responsible for the aqueous diffusion layer as well as the backward diffusion from the tissue to the lumen. The systemic transport of salicylic acid after intra-cheek-pouch adminis-

tration could be well approximated by the first-order absorption model including a lag-time process and the absorption rate constant estimated at pH 3.0 was about 5 times larger than that of at pH 4.0 (Table III). Similar results were obtained from the time course study of salicylic acid absorption (Fig. 3). However, since the pH change of the tissue adjacent to the blood capillary appeared to be negligible (though this determination has not been accomplished as yet), the real transfer rate constant from the tissue to the systemic circulation is assumed to be independent of the luminal pH. Taking into account the larger tissue accumulation of this compound at pH 3.0 in comparison with that of at pH 4.0 (Fig. 3c), the larger absorption rate constant from the lumen to the systemic circulation obtained at pH 3.0 might be due to the faster transfer or the higher partition to the tissue caused by the lower degree of ionization at pH 3.0 than at pH 4.0.

As to the effect of molecular weight on the absorption from the oral cavity, Siegel *et al.* reported that the permeability coefficients of short chain-length alcohols decreased from methanol to propanol and then increased to octanol, though the olive oil-to-water partition coefficients increased simply with increasing the chain-length.¹⁰⁾ They concluded from the *in vitro*¹¹⁾ and the *in vivo*¹⁴⁾ experiments that the absorption pathway of oil-soluble compounds is a transmembrane route whereas water-soluble molecules with a molecular volume of less than 80 cm³/mol cross primarily through membrane pores and larger water-soluble molecules pass extracellularly. In the present study, a more favourable correlation could be obtained between the absorption rates and the lipophilic indexes when the molecular-weight factor was taken into account. This is one of the reasons why the absorption from the keratinized hamster cheek pouch is explained by the passive diffusion mechanism.

A new experimental method using a hamster cheek pouch proposed in this paper is an available method for studying both the absorption and the following transfer processes to the systemic circulation. This will enable us to investigate the nature of the absorption from the keratinized oral mucosa in various severe experimental conditions which are never applied to human volunteers in the buccal absorption test. Absorp-

tion characteristics through the keratinized oral mucosa may be clarified by this hamster-cheek-pouch method in the near future.

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