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# Salivary Excretion of 5-Fluorouracil. I. Fluctuation of the Saliva/Plasma Concentration Ratio and Salivary Clearance in Beagle Dogs Following Bolus Intravenous Administration[1)]

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Salivary excretion of 5-fluorouracil (5-FU) was investigated following bolus intravenous administration (20 mg/kg) in four beagle dogs. Parotid (Pr) and mandibular-sublingual saliva (MS) were collected separately by stimulating salivation with 10% citric acid.

Significant correlations were observed between 5-FU concentrations in plasma and in each saliva ($p<0.01$). However, the saliva/plasma drug concentration ratios (S/P ratios) showed fairly large fluctuations ($0.472\pm0.303$ for Pr, $0.200\pm0.196$ for MS) and were not influenced by protein concentration in saliva or salivary flow rate. The observed S/P ratios were significantly different from the S/P ratios calculated by the use of Matin's equation which employed the observed salivary pH values and free fraction of 5-FU in plasma and saliva ($p<0.001$). Therefore, it was concluded that the salivary excretion of 5-FU cannot be explained by pH-partition theory.

Total salivary clearance of 5-FU ($0.123\pm0.093$ ml/min/kg) was less than 0.5% of total body clearance. In each saliva, salivary clearance of 5-FU was almost independent of salivary flow rate. The relationship between salivary clearance and plasma 5-FU concentration is discussed.

**Keywords**—5-fluorouracil; salivary drug excretion; salivary drug concentration; salivary pH; salivary protein binding; salivary flow rate; salivary clearance

Since the development of sensitive and convenient analytical methods such as enzyme immunoassay, *etc.*, therapeutic drug monitoring in saliva has been studied more extensively in the field of clinical pharmacology, and several review articles have been reported.[2–6)] Among those there have been a few reviews dealing with salivary excretion of some drugs pharmacokinetically.[5,6)] However, Matin's equation based on pH-partition theory is the only theoretical explanation so far proposed to explain the mechanisms of salivary drug excretion.[7)]

In the previous papers, salivary excretion of indomethacin[8)] and phenobarbital,[9)] and urea[10)] as a model compound, was investigated in dogs which had permanent fistulae for collecting parotid (Pr) and mandibular-sublingual saliva (MS) separately. In each case, the concentrations of the agent in plasma and in each saliva showed a good linear correlation. However, comparison of the observed saliva/plasma concentration ratios (S/P ratios) with those calculated by the use of Matin's equation demonstrated that salivary excretion of indomethacin and phenobarbital was explained not quantitatively but only qualitatively by pH-partition theory. Furthermore, it appeared that protein binding in saliva (usually assumed to be negligible) may influence the S/P ratio of phenobarbital, and it was suggested that the observed difference in salivary transport of urea in Pr and in MS might be associated with a difference in salivary transport of $Na^+$.

5-Fluorouracil (5-FU), and antineoplastic agent with low molecular weight, is extensively used in the palliative treatment of various solid tumors. Many clinical pharmacological examinations of 5-FU have been made in patients, and excretion of the drug in parotid saliva

following intravenous administration of 5-FU was reported in rats[11] and in patients.[12]

In the present work, salivary excretion of 5-FU was investigated following bolus intravenous administration in beagle dogs. Two different sources of saliva were collected separately. Simultaneously, salivary flow rate, salivary pH, and protein concentration in saliva were determined, and their effects on the S/P ratios were investigated in detail. Then, salivary clearance of 5-FU was calculated and compared with total body clearance. The mechanism of salivary excretion of 5-FU is discussed.

## Experimental

**Materials**——5-FU and 5-chlorouracil were kindly supplied by Kyowa Hakko Kogyo Co., Ltd., Tokyo, Japan, and Otsuka Pharmaceutical Factory, Inc., Naruto, Japan, respectively. For intravenous administration, a commercial preparation of 5-FU for injection was obtained from Kyowa Hakko Kogyo Co., Ltd. All other reagents and solvents were commercial products of analytical grade.

**Apparatus**——A high-performance liquid chromatographic (HPLC) system (Japan Spectroscopic Co., Ltd., Hachioji, Japan) was used, consisting of a TRIROTAR-II solvent delivery system, a model VL-611 variable loop injector, and a UVIDEC-100-III variable-wavelength ultraviolet absorbance detector operated at 266 nm, which was the maximum absorption wavelength for 5-FU under the present analytical conditions (described below). The detector was connected with a CHROMATOPAC C-R1A data module (Shimadzu Co., Ltd., Kyoto, Japan).

The separations were achieved by utilizing a 250 cm × 4.6 mm (i.d.) column packed with reverse-phase Fine SIL $C_{18}T$ (particle size 10 $\mu$m, Japan Spectroscopic Co., Ltd.) at room temperature. The mobile phase was $KH_2PO_4$ buffer (50 mM, pH 3.0) which had been filtered through a membrane filter (pore size 0.45 $\mu$m) and degassed, and the flow rate was set at 1.7 ml/min.

**Animals**——Three male and one female beagle dogs weighing 8.5—12.5 kg were employed without fasting. All beagle dogs had permanent fistulae[8] for collecting Pr and MS separately.

**Drug Administration and Sampling of Biological Fluids**——Dogs were given 20 mg/kg of 5-FU into the cephalic vein. Blood samples (about 3 ml) were withdrawn periodically into ice-chilled heparinized tubes. The plasma was obtained by centrifugation of the blood sample at 3000 rpm for 15 min. Saliva samples were taken simultaneously, and the midpoint of a sample collection period was regarded to as the sampling time. Saliva flow was stimulated with 10% citric acid, and saliva was collected under a liquid paraffin layer (about 3 ml) by using the method described in the previous paper.[9]

**Measurement of Salivary Flow Rate, Salivary pH and Protein Concentration in Plasma and Saliva**——Salivary flow rate was determined from the weight differences in the preweighed collection tubes containing liquid paraffin. The specific gravity of saliva was assumed to be approximately 1.00.[9] Salivary pH was measured with a combined electrode immersed through the liquid paraffin layer. Protein concentrations in plasma and saliva were determined by the method of Lowry *et al.*[13] using bovine plasma albumin (Fraction V, Sanko Pure Chemical Industries Co., Ltd., Tokyo, Japan) as a standard.

**Measurement of Protein Binding of 5-FU in Dog Plasma and Saliva**——The protein binding of 5-FU in beagle dog plasma and saliva was measured by equilibrium dialysis at three 5-FU levels (1.0, 10.0, and 50.0 $\mu$g/ml for plasma; 0.5, 10.0, and 50.0 $\mu$g/ml for saliva). A 2 ml sample was placed in a sac of seamless cellulose tubing (Type 8/32, 14 cm, Visking Co., Chicago, Ill., U.S.A.), which had been boiled twice for 30 min in distilled water and rinsed well with distilled water. The sacs were tied and put in 10 ml test tubes containing 2.5 ml of isotonic phosphate buffer (pH 7.4). The tubes were sealed tightly and incubated in a constant-temperature water bath kept at 37 °C for 24 h.

**Determination of 5-FU**——5-FU in dog plasma, saliva, and the solution outside the equilibrium dialysis sac was determined by HPLC by a slight modification of the methods of Christophidis *et al.*[14] and Driessen *et al.*[15] A 1 ml sample was added to the tube containing 25 $\mu$g of 5-chlorouracil as an internal standard and mixed well. Then 100 $\mu$l of 1 M sodium acetate buffer (pH 4.8 for plasma and MS, pH 4.3 for Pr and the solution outside the dialysis sac) and 0.5 ml of 20% anhydrous sodium sulfate solution were added. The mixture was extracted with 12 ml of ethyl acetate by shaking for 10 min and subsequent centrifugation at 3000 rpm for 10 min. The extraction was repeated with 10 ml of ethyl acetate by the same procedure. The combined ethyl acetate layer was evaporated to dryness under a dry nitrogen stream at about 50 °C. The extraction residue was dissolved in 0.5 or 1.0 ml of distilled water and washed with 1.0 ml of hexane twice for plasma or once for other samples. A 25 $\mu$l aliquot was injected into the liquid chromatograph. The quantitation was based on the 5-FU/5-chlorouracil peak area ratio.

## Results and Discussion

### Relationship between 5-FU Concentrations in Plasma and Saliva

5-FU concentrations in plasma, Pr, and MS after bolus intravenous administration of

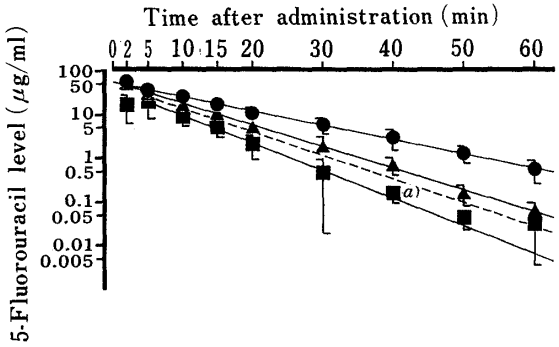


Fig. 1. Plasma and Saliva Levels of 5-Fluorouracil Following Bolus Intravenous Administration of 20 mg/kg in Four Beagle Dogs

●, plasma; ▲, parotid saliva (Pr); ■, mandibular-sublingual saliva (MS). Each point with vertical bar represents the mean value ± S.D. [*a*) for three beagle dogs]. The solid lines for plasma, Pr, and MS, and the broken lines for total saliva are the computer-fitted curves [Weight(i) = $1/C_i^2$, where $C$ is the 5-fluorouracil level].

TABLE I. Pharmacokinetic Parameters for 5-Fluorouracil Following Bolus Intravenous Administration of 20 mg/kg in Four Beagle Dogs (Value for Parameter ± S.D.)

| Parameters | Plasma ($n=36$) | Pr[a] ($n=36$) | MS[b] ($n=31$) | Total saliva ($n=29$) |
|---|---|---|---|---|
| $C_0$ (μg/ml) | 57.0 ±6.70 | 55.9 ±9.3 | 46.8 ±17.8 | 39.1 ±10.3 |
| $k$ (min$^{-1}$) | 0.0871 ± 0.0039 | 0.133 ± 0.006 | 0.212 ± 0.020 | 0.138 ± 0.009 |
| $t_{1/2}$ (min) | 7.95 ±0.36 | 5.20 ±0.24 | 3.26 ± 0.31 | 5.04 ± 0.34 |

All data weights (i) = $1/C_i^2$. *a*) Parotid saliva. *b*) Mandibular-sublingual saliva.

TABLE II. Correlation between Saliva and Plasma 5-Fluorouracil Concentrations Following Bolus Intravenous Administration of 20 mg/kg in Four Beagle Dogs

| | Pr | MS | Total saliva |
|---|---|---|---|
| Number of data points | 36 | 31 | 29 |
| Correlation coefficient | 0.974[a] | 0.887[a] | 0.965[a] |
| Regression line[b] | $Y=0.851X-2.21$ | $Y=0.523X-2.16$ | $Y=0.633X-2.08$ |

*a*) Significant ($p<0.01$). *b*) $X$ and $Y$ represent plasma and saliva 5-fluorouracil concentrations, respectively.

20 mg/kg in four beagle dogs are shown in Fig. 1. The drug concentrations in the three biological fluids decreased monoexponentially with time except in the case of MS at 2 min after administration. This exception may be because the 5-FU concentration in MS might not reach equilibrium with that in plasma within 2 min and/or that preliminary stimulation for salivation might not be sufficient so that these MS samples included saliva already present in the duct before the administration of 5-FU. Therefore, MS data at 2 min after administration of 5-FU were not used for the subsequent data analysis and discussion.

The determined plasma and saliva levels of 5-FU were fitted to the following equation, $C=C_0\exp(-kt)$, where $C$ and $C_0$ are the drug concentrations in plasma or saliva at time $t$ and $t=0$, respectively, and $k$ is the apparent first-order elimination rate constant. The drug concentrations in total saliva were calculated from both concentrations and flow rates for Pr and MS, and were also fitted to the same equation. The pharmacokinetic parameters were estimated by using the nonlinear least squares microcomputer program MULTI.[16] The most probable calculated regression lines are shown by solid lines for plasma, Pr, and MS and by a broken line for total saliva in Fig. 1, and the corresponding parameter values are summarized in Table I. It appeared that 5-FU was eliminated very rapidly from both plasma and saliva.

TABLE III. Saliva/Plasma 5-Fluorouracil Concentration Ratios, Salivary pH, Flow Rate, and Protein Concentrations Following Bolus Intravenous Administration of 20 mg/kg in Four Beagle Dogs (Mean ± S.D.)

| | Pr | MS | Total saliva |
|---|---|---|---|
| S/P ratio | 0.472 ± 0.303[a] | 0.200 ± 0.196 | 0.299 ± 0.204 |
| | $n=36$ | $n=31$ | $n=29$ |
| pH | 8.10 ± 0.06[a] | 7.98 ± 0.07 | |
| | $n=34$ | $n=31$ | |
| Flow rate | 0.099 ± 0.026 | 0.099 ± 0.020 | 0.201 ± 0.038 |
| (ml/min/kg) | $n=34$ | $n=31$ | $n=30$ |
| Protein concn. | 1.93 ± 1.48 | 1.71 ± 0.86 | 1.77 ± 0.92 |
| (mg/ml) | $n=35$ | $n=32$ | $n=29$ |

*a*) Significantly different from the value for MS at $p<0.001$.

TABLE IV. Comparison of the Coefficients of Variation in Saliva/Plasma Drug Concentration Ratios[a]

| Drugs | Pr | MS | Total saliva |
|---|---|---|---|
| 5-Fluorouracil | 64.1 | 98.0 | 68.1 |
| Indomethacin | 45.9 | 54.5 | —[b] |
| Phenobarbital | 19.0 | 11.5 | 11.5 |
| Urea | 16.0 | 25.8 | 15.4 |

*a*) Saliva was collected by stimulation with 10% citric acid following intravenous administration of drugs. *b*) Not determined.

The plasma biological half-life took the value of 7.95 ± 0.36 min (mean ± S.D.), which was slightly shorter than those for human[17] and rat.[11]

The relationship between 5-FU concentrations in plasma and in each saliva was examined by using linear regression analysis and comparing the saliva/plasma drug concentration ratios (S/P ratios). The coefficients of correlation and linear regression equations for each saliva are shown in Table II, and the S/P ratios are summarized in Table III together with some possible factors which may influence the drug concentration in saliva. Highly significant correlations were found between 5-FU concentrations in plasma and in each saliva ($p<0.01$) over a wide range of drug concentration in the biological samples. Similar significant correlations were observed for the data from the individual beagle dogs ($p<0.01$). The slope of the linear regression line for Pr was significantly larger than that for MS ($p<0.05$). This result is consistent with the finding that the S/P ratios for Pr were significantly higher than those for MS ($p<0.001$). Each regression line for saliva had a negative intercept. This result means that the S/P ratio of 5-FU increased with drug plasma concentration, and also reflects the fact that the biological half-life of 5-FU in plasma was longer than that in each saliva (Fig. 1, Table I).

The S/P ratios for both salivas showed considerable fluctuation. The coefficients of variation in the S/P ratios were calculated for each saliva and are shown in Table IV together with the results for other drugs reported previously.[8-10] It was found in each sort of saliva that the S/P ratios of 5-FU showed the largest fluctuation among these drugs. To investigate this fluctuation of the S/P ratios for 5-FU in more detail, some possible factors summarized in Table III were examined as follows.

## S/P Ratios and Salivary pH

Matin *et al.*[7] have proposed that for a weak acid, the S/P ratio ($R$) is predicted by the following equation based on pH-partition theory,

$$R=\frac{1+10^{(pH_S-pK_a)}}{1+10^{(pH_P-pK_a)}}\cdot\frac{f_P}{f_S} \quad (1)$$

where $pH_S$ = pH of saliva, $pH_P$ = pH of plasma, $pK_a$ = $pK_a$ value of the drug, $f_P$ = free fraction of drug in plasma, and $f_S$ = free fraction of drug in saliva. An increase of salivary pH results in a higher S/P ratio. Therefore, it appeared that the previously mentioned glandular difference in S/P ratio of 5-FU observed following bolus intravenous administration (Table III) could be explained by the glandular difference in salivary pH, which is significantly higher in Pr than in MS ($p<0.001$). However, no significant correlation was observed between salivary pH and S/P ratio in the two salivas (Pr: $r=-0.164$, $n=34$, $p>0.10$. MS: $r=-0.240$, $n=30$, $p>0.10$).

The observed S/P ratios of 5-FU were also compared with the S/P ratios calculated according to Eq. 1 as shown in Table V. In the calculation, the reported value of 8.0[18] was used

Table V. Comparison of Observed and Calculated Values for Saliva/Plasma 5-Fluorouracil Concentration Ratios (Mean ± S.D.)

| | Pr | MS |
|---|---|---|
| Observed value | 0.473 ± 0.303<br>$n=36$ | 0.200 ± 0.196[a]<br>$n=31$ |
| Calculated value[b] | 2.05 ± 0.146[c]<br>$n=34$ | 1.59 ± 0.130[a,c]<br>$n=31$ |

*a*) Significantly different from the value for Pr at $p<0.001$. *b*) Calculated by the use of Matin's equation; see the text for further details. *c*) Significantly different from the corresponding observed value at $p<0.001$.

as the $pK_a$ of 5-FU, and $pH_P$ was fixed at 7.4. The salivary pH values that were measured in individual samples were employed. For protein binding of 5-FU, Celio *et al.*[11] have reported that $f_P$ in rats was $>0.9$, as determined by equilibrium dialysis, but no data on $f_S$ have been reported for this drug. In this study, $f_P$ and $f_S$ in dogs were measured by equilibrium dialysis, and the free fractions did not show any significant dependence on the 5-FU concentration. The mean values with the standard deviation were 0.935 ± 0.013 ($n=6$) for $f_P$, 0.829 ± 0.032 ($n=6$) for $f_S$ in Pr and 0.878 ± 0.048 ($n=6$) for $f_S$ in MS. These mean values were used in the calculation. The glandular difference in the calculated S/P ratios of 5-FU corresponded with that in the observed S/P ratios. However, the calculated S/P ratios were significantly higher than the corresponding observed S/P ratios (for both saliva, $p<0.001$), and the calculated values for Pr and MS were about four and eight times larger than the observed values, respectively. No significant correlation was found between the observed and calculated S/P ratios of 5-FU in the two salivas (Pr: $r=-0.160$, $n=34$, $p>0.10$. MS: $r=-0.208$, $n=30$, $p>0.10$). Therefore, it was concluded that salivary excretion of 5-FU could not be quantitatively explained by pH-partition theory.

## S/P Ratio and Protein Binding

Though the protein binding of drugs in saliva is usually considered to be negligible, *i.e.* $f_S=1$, it was reported that protein binding of phenobarbital in saliva should be taken into account as well as salivary pH.[9] Since it was impossible to determine $f_S$ and $f_P$ for all sets of saliva and plasma samples following bolus intravenous administration of 5-FU, the relationship between the S/P ratio and protein concentration in saliva instead of $f_S$ was investigated.

No significant difference could be found between the protein concentrations in Pr and MS (Table III). Though the scatter diagram is omitted, the correlation coefficients between the S/P ratio of 5-FU and protein concentration in Pr, MS, and total saliva were 0.553 ($n=35$, $p<0.01$), 0.026 ($n=31$, $p>0.01$), and 0.372 ($n=28$, $p>0.10$), respectively. They were not significant or were significant but very low. Thus it was difficult to attribute the fluctuation of the S/P ratio for 5-FU to that of protein concentration in saliva.

**S/P Ratio and Salivary Flow Rate**

Salivary flow rate may be considered to be one of the factors which influence the S/P ratio of drugs, since Mucklow *et al.* have reported that the S/P ratio of tolbutamide increased with the salivary flow rate and pH in man.[3] Dawes and Jenkins concluded that salivary pH increased depending on the salivary flow rate and was independent of the kind of stimulus of salivary secretion in man.[19] Under the conditions of the present study in beagle dogs, however, such a distinct relationship was not observed between salivary pH and flow rate for Pr ($r=0.054$, $n=33$, $p>0.05$), while salivary pH for MS tended to decrease in two beagle dogs and to increase in two other beagle dogs with salivary flow rate. Therefore, as in the case of intravenous administration of phenobarbital,[9] the effects of salivary flow rate on the S/P ratio of 5-FU could be discussed independently of salivary pH in this study.

No significant correlation was found between the S/P ratio of 5-FU and the flow rate for each saliva (Pr: $r=0.111$, $n=34$, $p>0.10$. MS: $r=0.098$, $n=30$, $p>0.10$. Total saliva: $r=0.100$, $n=29$, $p>0.10$). No difference was observed in salivary flow rate between Pr and MS following bolus intravenous administration of 5-FU (Table III). Consequently, it might be concluded that salivary flow rate had no influence on the S/P ratio of 5-FU under the conditions in the present study.

**Salivary Clearance**

In the previous paper,[10] the concept of salivary clearance ($CL_S$) was introduced for stimulated salivary excretion to discuss the salivary excretion of drugs from a kinetic point of view. The salivary clearance of 5-FU was calculated by use of the following equation,

$$CL_S=\frac{C_S \times V_S}{C_P} \tag{2}$$

where $C_S$ and $C_P$ are the drug concentrations in saliva and plasma, respectively, and $V_S$ is the salivary flow rate. The estimates are listed in Table VI together with those[10] for phenobarbital and urea. Total salivary clearance was calculted by doubling the sum of $CL_S$ for Pr and $CL_S$

TABLE VI. Comparison of Salivary Clearance with Total Body Clearance[a]

| | | Drugs | | |
|---|---|---|---|---|
| | | 5-Fluorouracil | Phenobarbital | Urea |
| Salivary clearance (ml/min/kg) | Pr | 0.044±0.030[b] $n=34$[c] | 0.106±0.020 $n=30$ | 0.074±0.034 $n=36$ |
| | MS | 0.021±0.020 $n=30$ | 0.115±0.026 $n=30$ | 0.056±0.023 $n=36$ |
| | Total | 0.123±0.099 $n=29$ | 0.442±0.092 $n=30$ | 0.30 ±0.24 $n=35$ |
| Total body clearance (ml/min/kg) | | 30.6 ±5.00 $N=36$[e] | 0.179±0.082[d] $N=44$ | 1.69 ±0.50[d] $N=12$ |

*a*) Saliva was collected by stimulation with 10% citric acid following intravenous administration of drugs. *b*) Mean±S.D. *c*) Number of observed data points. *d*) Mean±S.E. (W. E. Deming, "Statistical Adjustment of Data," John Wiley and Sons, Inc., New York, 1946). *e*) Number of input data.

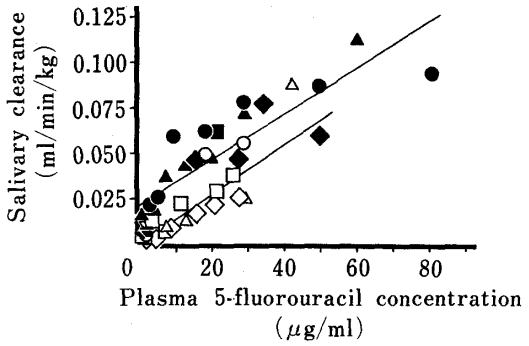


Fig. 2. Relationship between Salivary Clearance and Plasma 5-Fluorouracil Concentration Following Bolus Intravenous Administration of 20 mg/kg in Four Beagle Dogs

Solid symbols: Pr ($r=0.881$, $n=25$, $p<0.01$, $Y=0.00126X+0.0211$). Open symbols: MS ($r=0.835$, $n=22$, $p<0.001$, $Y=0.00139X$). Each shape of symbol represents an individual dog. Plotted data points are those with salivary flow rates ranging from the mean value plus one S.D. to the mean value minus one S.D.

for MS, since permanent fistulae for collecting Pr and MS separately were only present in one of each pair of salivary glands. Salivary excretion of phenobarbital may make a profound contribution to the total body clearance in the case of continuously stimulated salivation in beagle dogs, because phenobarbital has a large total salivary clearance.[10] For 5-FU, however, total salivary clearance was less than 0.5% of total body clearance. This result indicates that salivary excretion of 5-FU following bolus intravenous administration in beagle dogs negligibly contributes to the elimination of the drug from the body even under conditions of stimulated salivation.

Since Eq. 2 can be rearranged to the following equation, $CL_S=(\text{S/P ratio})\times V_S$, salivary clearance is expected to be related direclty to salivary flow rate provided that the S/P ratio is constant. In fact, it has been found that there were very high correlations between salivary clearance of urea and salivary flow rate in both Pr and MS.[10] However, in this experiment no significant correlations were observed in MS ($r=0.277$, $n=30$, $p>0.10$) and total saliva ($r=0.290$, $n=29$, $p>0.10$), although a significant but low correlation was found in Pr ($r=0.432$, $n=34$, $p<0.02$). These observations seemed to be consistent with the marked variability in the S/P ratio of 5-FU for each saliva (Tables III and VI).

The relationship between salivary clearance and plasma 5-FU concentration is illustrated in Fig. 2. It was difficult to examine the relationship at a constant salivary flow rate because of the insufficient number of data points. Therefore, data points corresponding to salivary flow rates ranging from the mean value plus one S.D. (standard deviation) to the mean value minus one S.D. were plotted in the figure. It should be noted that the salivary clearances for both Pr and MS were not constant but increased with plasma 5-FU concentration. This result is consistent with the large fluctuation of the S/P ratio for 5-FU in each saliva and may be considered to be caused by the contribution of some active process, such as is known to occur in the absorption of 5-FU from the small intestine in rats,[20] as well as the contribution of the passive process in the salivary excretion of 5-FU, or by impairment of the salivary glands by 5-FU. There is a possibility that the active reabsorption process in salivary excretion of 5-FU is saturated by a mechanism similar to that suggested by Jusko and Levy in regard to the renal excretion of riboflavin in man and dog.[21] On the other hand, some cytological change in the salivary glands which was reported in mice following sublethal doses of 5-FU[22] may affect the salivary excretion of 5-FU following bolus intravenous administration of 20 mg/kg in beagle dogs.

Celio *et al.*[11] have reported that the S/P ratio of 5-FU for Pr tended to decrease at higher doses in rats and they suggested that parotid salivary excretion of 5-FU might be affected by back-diffusion, ion trapping, alterations in blood supply, and damage of the parenchyma of the gland. However, it is difficult to compare the results obtained in the present study with their results, since they carried out the experiments in rats during pilocarpine infusion to stimulate salivation and, furthermore, they did not collect periodical saliva samples which could be related to the blood samples. In order to clarify the reason why the S/P ratios of 5-

FU in each saliva show large fluctuations and why the salivary clearances for both Pr and MS are not constant with varying plasma 5-FU concentration, it may be necessary to carry out experiments with a wider range of plasma 5-FU concentrations or by infusing 5-FU at a constant rate, and such studies are in progress in this laboratory.

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**References and Notes**

1) This paper constitutes Part IV of the series entitled "Salivary Excretion of Drugs." Part III: J. Watanabe, S. Mizuno, N. Masuda, Y. Hayashi, K. Iwamoto, J. Hirate, and S. Ozeki, *J. Pharmacobio-Dyn.*, **7**, 294 (1984).
2) M. Danhof and D. D. Breimer, *Clin. Pharmacokinet.*, **3**, 39 (1978).
3) J. C. Mucklow, M. R. Bending, G. C. Kahn, and C. T. Dollery, *Clin. Pharmacol. Ther.*, **24**, 563 (1978).
4) J. C. Mucklow, *Ther. Drug Monit.*, **4**, 229 (1982).
5) J. Posti, *Pharm. Acta Helv.*, **57**, 83 (1982).
6) J. Zuidema and C. A. M. von Ginneken, *Pharm. Acta Helv.*, **58**, 88 (1983); *idem, ibid.*, **58**, 136 (1983).
7) S. B. Matin, S. H. Wan, and J. H. Karam, *Clin. Pharmacol. Ther.*, **16**, 1052 (1974).
8) J. Watanabe, Y. Urasaki, Y. Nakase, H. Ueda (née Kiyohara), K. Iwamoto, and S. Ozeki, *J. Pharmacobio-Dyn.*, **4**, 336 (1981).
9) J. Watanabe, Y. Nakase, Y. Urasaki, Y. Hayashi, K. Iwamoto, and S. Ozeki, *J. Pharmacobio-Dyn.*, **4**, 968 (1981).
10) J. Watanabe, S. Mizuno, N. Masuda, Y. Hayashi, K. Iwamoto, J. Hirate, and S. Ozeki, *J. Pharmacobio-Dyn.*, **7**, 294 (1984).
11) L. A. Celio, G. J. DiGregorio, E. Ruch, J. N. Pace, and A. J. Piraino, *J. Pharm. Sci.*, **72**, 597 (1983).
12) L. A. Celio, G. J. DiGregorio, E. Ruch, J. Pace, and A. J. Piraino, *Eur. J. Clin. Pharmacol.*, **24**, 261 (1983).
13) O. H. Lowry, N. J. Rosebrough, A. L. Farr, and R. J. Randall, *J. Biol. Chem.*, **193**, 265 (1951).
14) N. Christophidis, G. Mihaly, F. Vajda, and W. Louis, *Clin. Chem.*, **25**, 83 (1979).
15) O. Driessen, D. De Vos, and P. J. A. Timmermans, *J. Chromatogr.*, **162**, 451 (1979).
16) K. Yamaoka, Y. Tanigawa, T. Nakagawa, and T. Uno, *J. Pharmacobio-Dyn.*, **4**, 879 (1981).
17) J. M. Collins, R. L. Dedrick, F. G. King, J. L. Speyer, and C. E. Myers, *Clin. Pharmacol. Ther.*, **28**, 235 (1980).
18) D. W. Newton and R. B. Kluza, *Drug Intell. Clin. Pharm.*, **12**, 546 (1978).
19) C. Dawes and G. N. Jenkins, *J. Physiol.*, **170**, 86 (1964).
20) L. S. Schanker and D. J. Tocco, *J. Pharmacol. Exp. Ther.*, **128**, 115 (1960); L. S. Schanker and J. J. Jeffery, *Nature* (London), **190**, 727 (1961); L. S. Schanker and D. J. Tocco, *Biochim. Biophys. Acta*, **56**, 469 (1962); L. S. Schanker and J. J. Jeffery, *Biochem. Pharmacol.*, **11**, 961 (1962).
21) W. J. Jusko and G. Levy, *J. Pharm. Sci.*, **59**, 765 (1970).
22) M. K. Kim and S. S. Han, *Proc. Soc. Exp. Biol. Med.*, **139**, 1246 (1972).