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Biopharmaceutical Study of Pyrithioxin following Oral and Intravenous Administration to Dog[1,2]

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After single oral and intravenous administration of pyrithioxin (I) to beagle dogs, the blood levels of 5′-desoxy-5′-methylsulfinylpyridoxol (II), one of the main metabolites of I in blood and urine, were measured with a high-performance liquid chromatography. The existence of I was not detected in blood samples for 24 hr after an oral administration of I at dose levels less than 100 mg of I/kg. But, it was detected in the first blood sample collected 1 min after intravenous injection at dose levels of 10 and 5 mg/kg, but not in the sample collected 3 min after injection. After oral and intravenous administrations of I, non-first-order disappearance of II in blood was observed. The results suggest a possible enterohepatic cycling of I-glucuronide (I-G). A study was made on the metabolites in the urine samples after two routes of administration, demonstrating the existence of I-G, II, and II-glucuronide (II-G). Likewise, II and II-G were found in the urine following oral and intravenous administrations of II·HCl. A total amount of II in mole percent of dose excreted in the urine after oral administration of I·2HCl filled in hard gelatin capsules decreased significantly with an increase of dose suggesting change of bioavailability with change of the dose. The results were seemed to be partially subjected to the poor absorbability at the intestinal tract owing to the low solubility of I at the physiological pH of 4—7.5.

Mucosal-to-serosal permeation of I across the everted rat intestine *in vitro* was found to proceed without metabolism during and after permeation. Thus, it may be considered that the metabolism of I takes place after absorption, mainly in the liver.

Keywords——pyrithioxin; oral administration; intravenous administration; beagle dog; absorption; urinary excretion

Pyrithioxin (I), 3,3′-(dithiodimethylene)bis[5-hydroxy-6-methyl-4-pyridinemethanol], has been clinically used as a "neurodynamic drug". Darge, Liss, and Oeff reported on the study of pharmacokinetics and metabolism of ^{35}S-labelled I in rat[4] and man.[5] Recently, Warner, Erdmann, and Thel[6] reported on an autoradiographic study on the distribution of ^{3}H-labelled I in monkey and mouse. They demonstrated the distribution of radioactivity in the brain and in other organs. But, little was reported on a quantitative assay of metabolites in blood and urine. The present report describes the blood concentration and urinary excretion profiles of I and its metabolites with the assay of a high-performance liquid chromatography (HPLC) following single oral or intravenous administrations to beagle dogs.

Experimental

Materials——I was obtained through the courtesy of the Chugai Pharmaceutical Co., Ltd. I dihydro-

1) Biopharmaceutics of Pyrithioxin. II. Part I: K. Kitao, N. Yata, and A. Kamada, *Chem. Pharm. Bull.* (Tokyo), **25**, 1335 (1977).
2) A part of this work was presented at the 96th Annual Meeting of Pharmaceutical Society of Japan, Nagoya, Apr. 1976.
3) Location: *Yamadakami, Suita, Osaka.*
4) W. Darge, E. Liss, and K. Oeff, *Arzneim.-Forsch.*, **19**, 5 (1969).
5) W. Darge, E. Liss, and K. Oeff, *Arzneim.-Forsch.*, **19**, 9 (1969).
6) G. Werner, G. Erdmann, and S. Thel, *Arzneim.-Forsch.*, **26**, 825 (1976).

chloride (I·2HCl) and 5′-desoxy-5′-methylsulfinylpyridoxol hydrochloride (II·HCl) were prepared by the reported procedures.[1)]

Drug Administration——Six adult beagle dogs weighing 8—12 kg (mean 10.3 kg) were used. They were kept fast about 12 hr prior to the experiment with water given *ad libitum*. They were not fed until 10 hr after the drug administration, but water was given *ad libitum* during the experiments. At least one week was allowed between drug administrations.

Oral Experiment——A single oral dose of I or I·2HCl, filled in hard gelatin capsules (Matsuya Co., Ltd. No. 0), was administered with 30 ml of water in a random crossover fashion. The dose levels were 50—100 mg/kg for I and 15.0—119.8 mg/kg for I·2HCl (equivalent to 12.5—100.0 mg of I/kg). For aqueous solution of I·2HCl, a dose of 59.9 mg/kg (equivalent to 50 mg of I/kg) dissolved into 30 ml of water was injected into the stomach through a rubber gastric tube immediately followed by 2 ml of flushing water.

Intravenous Experiment——I has a very low water solubility at physiological pH except for that of gastric juice. Thus, I·2 HCl was put into solution in propylene glycol at a concentration of 59.9 mg/ml. The experiments were performed at two dose levels, 0.1 and 0.2 ml/kg (equivalent to 5 and 10 mg of I/kg, respectively). The experiments with II·HCl were performed employing aqueous solution at a concentration of 58.5 mg/ml with dose levels of 0.1 and 0.2 ml/kg (equivalent to 5 and 10 mg of II/kg, respectively). The drug solutions were injected into the cephalic vein of either forefoot with a constant rate for 1 min.

Blood and Urine Samples——Fifteen blood samples were collected for 30 hr in oral experiments and for 6 hr in intravenous experiments. Control blood samples for all experiments were drawn from the jugular vein just prior to the administration of the drug. Blood samples of 5 ml were drawn from the jugular vein of either side with a 5 ml disposable syringe, previously flushed with 10% EDTA solution, using a 32 mm, 22-gauge needle. Urine samples were collected from 0 to 48 hr in a 1 liter glass reservoir which was kept at 2° with ice. The volume of urine at each scheduled collection was recorded. After mixing and filtering, a sample of approximately 5 ml was placed into a glass-stoppered test tube. The blood and urine samples were stored in a refrigerator until analyzed. The details of the handling of blood and urine samples, analysis with HPLC, and caluculation of mole percent excretion have been previously described.[1)]

Transfer of I across Everted Rat Intestine——Intestinal transfer of I was studied with a modified method of Crane and Wilson.[7)] Sprague-Dawley strain rats, weighing approximately 300 g, were kept fasting 12 hr prior to the experiment. Water was allowed *ad libitum*. After decapitation and severing the intestine at the pyloric junction, the first 15 cm of the intestine was discarded, the gut was washed with Krebs-Ringer's solution with 0.3% glucose (pH 7.4) (K-R solution), and was everted. The proximal portion was devided into two 15 cm segments. The segments were placed into test tubes containing approximately 90 ml of K-R solution (mucosal solution). Three ml of K-R solution was placed inside each sac (serosal solution). The solutions were kept at 37° under continuous bubbling with oxygen-carbon dioxide (95: 5, v/v). After equilibrating for 10 min, ten ml of propylene glycol solution of I·2HCl (2 mmoles/liter) was added into the mucosal solution. The concentrations of I in each solution were measured after the 1 hr experiment with the method of HPLC described previously.[1)] The existence of metabolites was studied with HPLC and thin-layer chromatography (TLC).

Solubility of I——The pH-profile of the solubility of I was measured at 37° employing phosphate buffers (μ=0.15) with a pH range from 3 to 8. The concentration of I was spectrophotometrically measured at 296 nm after acidifying the solution with 0.5 N HCl.

Results and Discussion

Oral Administration of I and I·2HCl

Following single oral administration of I·2HCl filled in hard gelatin capsules at dose levels of 119.8 and 59.9 mg/kg (equivalent to 100 and 50 mg of I/kg, respectively) to six dogs, the mean blood concentration of II *versus* time (BCT) curves are shown in Fig. 1. I and I-glucuronide (I-G) were not detected in the blood samples by HPLC like the case with TLC. Each curve was composed of two regions: the initial peak appeared 1—1.5 hr and the second peak 5—8 hr after administration. The maximum blood levels of II were 5.4 and 4.1 μg/ml and the areas under the BCT curve (AUC) were 56.7 and 43.0 μg·hr/ml for large and small doses, respectively. The AUC was obtained connecting the plots with straight lines following a trapezoidal method. The values of specific AUC, AUC per dose (mg/kg), were 0.8565 ± 0.0920 and 0.5879 ± 0.1496 $\mu g \cdot hr \cdot ml^{-1} \cdot mg^{-1} \cdot kg$ for small and large doses, respectively (significant at the level of 99%). Thus, it may be considered that the second peak of the BCT curve is probably due to enterohepatic cycling of I and/or its metabolites

7) R.K. Crane and T.H. Wilson, *J. Appl. Physiol.*, **12**, 145 (1958).

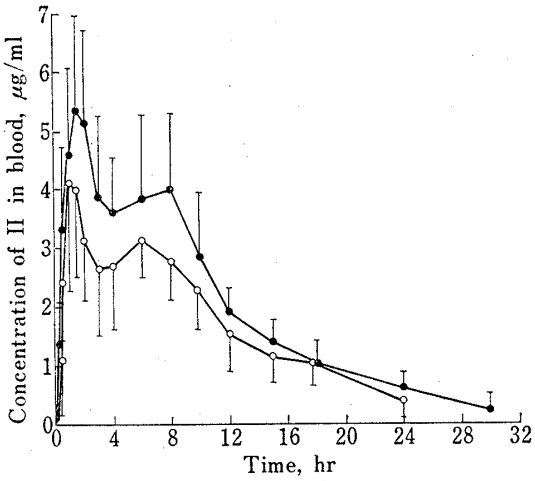

Fig. 1. Mean Blood Concentration of II after Single Oral Administration of I·2HCl to Six Dogs

key: ○, 59.9 mg/kg; and ●, 119.8 mg/kg
Each point represents the mean and bars indicate SD.

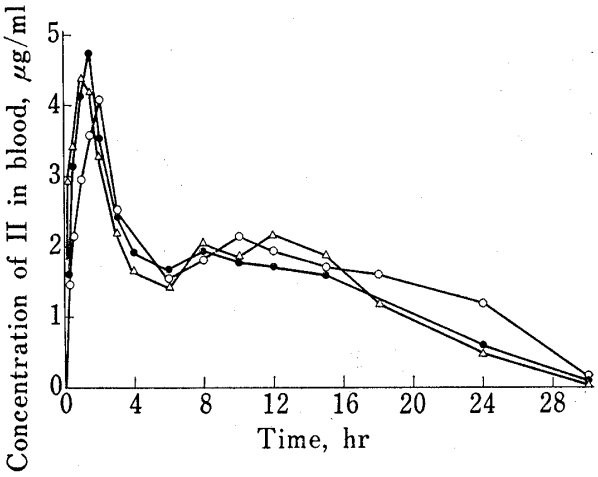

Fig. 2. Blood Concentration of II after Single Oral Administration of I and I·2HCl (Dose: Equivalent to 100 mg of I/kg)

key: △, I·2HCl solution; ●, I·2HCl capsule; and ○, I capsule

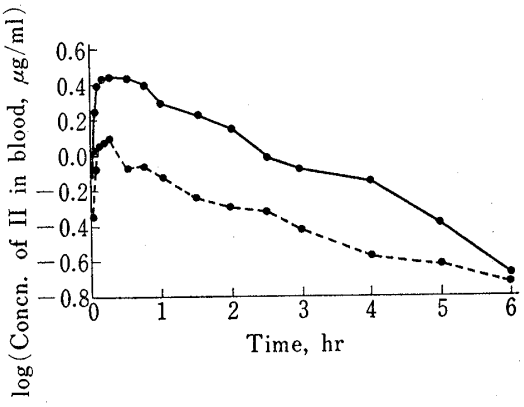

Fig. 3. Blood Concentration of II after Intravenous Injection of I·2HCl

key: ---●---, 5.99 mg/kg; and —●—, 11.98 mg/kg

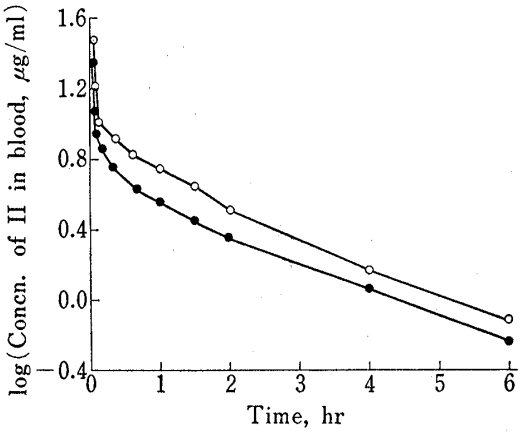

Fig. 4. Blood Concentration of II after Intravenous Injection of II·HCl

key: ●, 5.85 mg/kg; and ○, 11.69 mg/kg

and that the change of the specific AUC with the dose is closely correlated with an incomplete absorbability being subjected to the poor solubility of I at physiological pH of intestine (Fig. 9) and/or the enterohepatic cycling.

Figure 2 shows typical BCT curves obtained following single oral administrations of I and I·2HCl filled in hard gelatin capsules and aqueous solution of I·2HCl at one dose level equivalent to 100 mg of I/kg to a dog. The initial maximum blood levels were observed in order of the degree of participation of dissolution processes, *i.e.*, 1, 1.5, and 2 hr after administration of aqueous solution of I·2HCl, I·2HCl in capsules, and I in capsules, respectively. But, no significant difference was observed between the three preparations with similar AUC values of 42.7 μg·hr/ml for aqueous solution of I·2HCl, 41.3 μg·hr/ml for I·2HCl in capsules, and 44.5 μg·hr/ml for I in capsules, suggesting a similar bioavailability of I in terms of AUC of II for the three preparations.

Intravenous Administration of I·2HCl

The BCT curves of II following intravenous administrations of propylene glycol solution of I·2HCl at dose levels of 11.98 and 5.99 mg/kg (equivalent to 10 and 5 mg

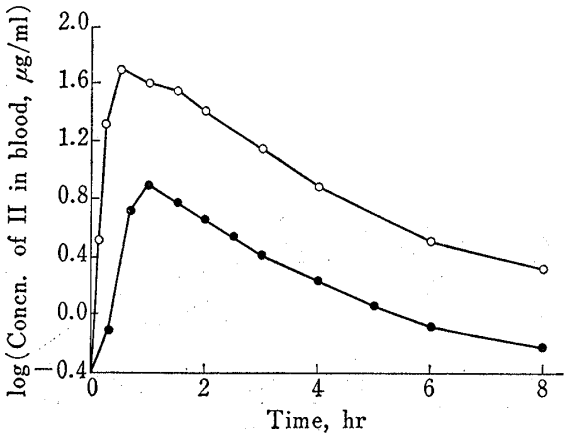

Fig. 5. Blood Concentration of II after Oral Administration of II·HCl

key: ●, 11.69 mg/kg; and ○, 58.5 mg/kg

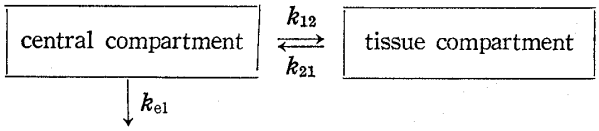

Fig. 6. Schematic Representation of the Body as a Two-Compartment Open Model

k_{12} is the transfer rate constant from the central compartment to the tissue compartment, k_{21} is the transfer rate constant from the tissue compartment to the central compartment, and k_{el} is the elimination rate constant of the drug.

TABLE I. Pharmacokinetic Parameters after Intravenous Injection of II·HCl

Parameters	Dose, mg/kg	
	5.85	11.69
Body wt., kg	10.5	11.3
k_{el}, hr^{-1}	1.11	0.955
$t_{1/2}$, hr	0.622	0.726
k_{12}, hr^{-1}	3.94	3.53
k_{21}, hr^{-1}	2.23	2.38
V_c, l	3.18	4.76
V_t, l	5.63	7.08

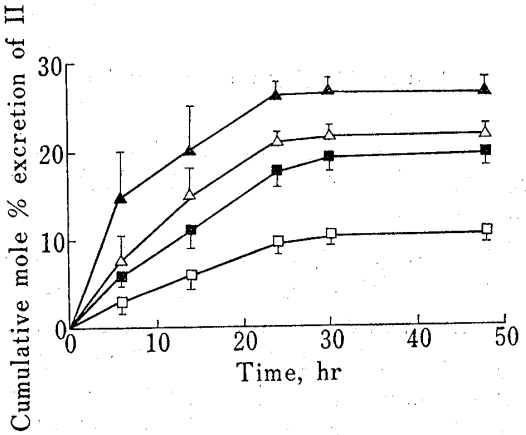

Fig. 7. Cumulative Mole Percent Excretion of II in Urine after Oral Administration of I·2HCl in Several Doses to Four Dogs

dose: equivalent to I, key: ▲, 12.5 mg/kg; △, 25.0 mg/kg; ■, 50.0 mg/kg; and □, 100 mg/kg
Each point represents the mean and bars indicate. SD.

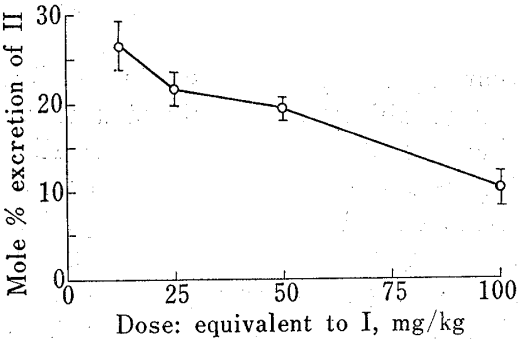

Fig. 8. Mole Percent Excretion of II in Urine after Single Oral Administration of I·2HCl to Four Dogs

Each point represents the mean and bars indicate ±SD.

of I/kg, respectively) are shown in Fig. 3. The existence of I in blood was demonstrated in the first blood sample collected 1 min after injection, but not in the blood sample at 3 min because of its rapid metabolism. Each semilogarithmic curve of II was composed of an initial portion with a rapid increase and irregular disappearance. The metabolite II reached its maximum blood level 10 min after injection.

Intravenous and Oral Administrations of II·HCl

The BCT curves of II following an intravenous administration of aqueous solution of II·HCl and an oral administration of II·HCl filled in hard gelatin capsules to a dog are presented in Fig. 4 and 5, respectively. Unlike intravenous and oral administrations of I·2HCl, apparent first-order disappearance was observed in the period of 1 to 6 hr for the intravenous administration and 2 to 6 hr for the oral administration. Thus, it may be considered that the atypical disappearance of II in blood after intravenous and oral administrations of I·2HCl is responsible for the non-first-order enterohepatic cycling of I–G. The semilogarithmic BCT curve after intravenous administration of II·HCl shows biexponential decay (Fig. 4). Thus, pharmacokinetic parameters of intravenous administration were estimated with a two-compartment open model (Fig. 6 and Table I). To discuss the effect dose in the administration of II·HCl, it is required to make further complehensive experiments. But, it was found that the blood concentration of II rapidly decreased with half-life of 40 min for two dose levels. It was interesting to note that the AUC value of oral administration at a dose levels of 11.69 mg/kg was 19.4 μg·hr/ml and that of intravenous administration at the same dose was 22.1 μg·hr/ml, suggesting an insignificant influence of the administration route on the availability of II·HCl.

The second peak which was observed in oral administration of I was not observed in oral administration of II·HCl at two dose levels. Thus, the appearance of the second peak for oral administration of I·2HCl was considered to be responsible for the enterohepatic cycling of I–G.

Urinary Excretion

The mean cumulative amounts of II excreted in the urine as a function of time were studied following single oral administrations of I·2HCl filled in hard gelatin capsules at the dose levels equivalent to 12.5—100.0 mg of I/kg to four dogs (Fig. 7). The excreted

Table II. Mole Percent Recovery of II in Urine after Single Oral Administration of Capsule, I·2HCl Capsule, and I·2HCl Solution to Four Dogs

Dose (equiv. to I)	I capsule	I·2HCl capsule	I·2HCl solution
50.0 mg/kg	20.2±6.0	19.0±1.0	20.3±3.3
100.0 mg/kg	12.3±5.4	10.5±1.9	11.2±4.1

Values reported are mean±SD for four dogs.

Table III. Mole Percent Excretion of Metabolites in Urine after Single Oral Administration of I·2HCl to Four Dogs (Dose: 59.9 mg/kg)

Metabolites	Mole % excretion
I–G	2.6±0.7
II	19.0±1.0
II–G	4.3±0.8
Total	25.9±1.7

Values reported are mean±SD for four dogs.

Table IV. Mole Percent Excretion of Metabolites in Urine after Single Intravenous Injection of I·2HCl to Four Dogs (Dose: 11.98 mg/kg)

Metabolites	Mole % excretion
I–G	8.8±1.4
II	24.0±1.4
II–G	9.4±1.2
Total	42.2±2.2

I was not detected in urine.
Values reported are mean±SD for four dogs.

amounts were presented in terms of mole percent of the dose of I·2HCl. In all cases, 90% of the total excretion was excreted in the urine within 24 hr after administration. The mole percent of recovery of II in the urine decreased with an increase of the dose of I (Fig. 8).

The influence of the dosage form and dose level on the urinary recovery of II was studied (Table II). No significant difference was observed between the three preparations with recovery values of about 20 and 11% for the small and large doses, respectively. The results were closely correlated to those of similar AUC values of the three preparations (Fig. 2) and strongly suggested that the difference in dissolution processes of I and I·2HCl in gastric juice and the initial delay of drug release by capsules were not significant for the apparent availability of the drug in terms of the urinary excretion of II.

The previous report[1] demonstrated that I–G, II, and II–G were found in the urine following an oral administration of I and its hydrochloride. Thus, mean mole percent of the recovery of each metabolite was measured employing acid hydrolysis of the urine and HPLC assay.[1] The results of an oral administration of I·2HCl at a dose level of 59.9 mg/kg filled in hard gelatin capsules to four dogs are presented in Table III. Similarly, the results of intravenous administration of propylene glycol solution of I·2HCl at a dose level of 11.9 mg/kg to four dogs are presented in Table IV. The excretion of I was not observed in the urine with HPLC and TLC in the experiments of oral and intravenous administrations of I·2HCl. The metabolites in the urine after oral and intravenous administrations of I·2HCl were II and small but never to be ignored in amounts of I–G and II–G. Although the bioavailability of I·2HCl is considered to be complete for intravenous administration, the total recovery was less than the dose. A possible existence of unknown metabolites should be considered but any other metabolites could not be found by TLC and HPLC. So, the poor recovery in the urine is considered to be subjected to a possible biliary excretion, poor reabsorbability of I and/or its metabolites and a possible excretion in feces.

Darge, *et al.*[4] studied on the excretion of ^{35}S following an oral administration of ^{35}S-labelled I·2HCl to rats and found more than 95% of the dose in the urine and feces with about 17% in feces.

Thus, it may be considered that for the complete understanding of pharmacokinetics of I, it is required to study the excretion of I and its metabolites in feces. But, in a

Table V. Mole Percent Excretion of Metabolites in Urine after Single Intravenous Injection of II·HCl to Four Dogs (Dose: 5.85 mg/kg)

Metabolites	Mole % excretion
II	32.5±1.9
II–G	7.0±1.5
Total	39.5±1.5

I was not detected in urine.
Values reported are mean±SD for four dogs.

Table VI. Permeation of I through Everted Sac of Rat Intestine at 37° for 1 hr

	Final Concn., μg/ml
Serosal side	11.9±3.1
Mucosal side	65.2±5.2

Values reported are mean±SD for 5 experiments.
Initial concentration of mucosal side is 73.7 μg/ml

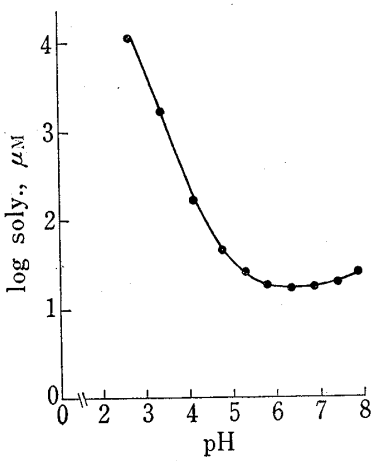

Fig. 9. pH-Profile of Solubility of I

preliminary experiment, it was found thet I was easily reduced to 5′-thiopyridoxol by *Escherichia coli*, which was abundant in the rectum and feces, revealing the difficulty of the pharmacokinetic study of I.

To study the disposition processes of I after absorption, II·HCl was intravenously administered at a single dose level of 5.85 mg/kg to four dogs (Table V). The total recovery of II in the urine was only 39.5% of the dose suggesting another possibility of biliary excretion of II and/or its glucuronide and their poor reabsorbability at the intestine. Thus, it may be considered that a pharmacokinetic analysis of the results of intravenous administration of II·HCl requires intricated experiments and is inadequate in analyzing on the basis of the two-compartment open model.

From the result of similar AUC values in terms of II for oral and intravenous administrations of II·HCl, it may be considered that the intestinal absorption of II is favorable after oral administration of II·HCl. But, from the result of poor recovery of II and II–G in the urine after intravenous administration of II·HCl, it is suggested that the reabsorbability of II–G after biliary excretion is insufficient. And the poor availability of I in terms of the excreted amounts of the metabolites in the urine is subjected to the poor reabsorbability of II–G as well as the low solubility of I in the intestinal fluid.

Permeation of 1 through Everted Rat Intestine

It was well established that I is easily metabolized and disposed by complicated processes of metabolism. To study the participation of metabolism to drug absorption, a modified method of Crane and Wilson[7] employing everted rat intestine was used (Table VI). The initial concentration of 73.7 μg of I·2HCl/ml in mucosal solution decreased to 65.2 μg/ml in the period of 1 hr at 37°. The concentration of I in serosal solution was 11.9 μg/ml. Metabolites of I were not found in either solutions within the detectable limits of HPLC and TLC.

Thus, it may be considered that the present results are obtained with *in vitro* experiments of the rat intestine, but a similar permeation of I is possible in the intestinal tract of dog. It may be concluded that the absence of I in blood samples following oral administration of I supports a rapid metabolism of I in the liver.

pH-Profile of Solubility of I

The pH-profile of water solubility of I was measured at 37° employing pH-adjusted phosphate buffer (Fig. 9). The pK_a values of I were reported as 3.6 and *ca.* 10 with a solubility method,[8] and 4.43 and 8.84 with a spectral method[9] for the hydroxyl group and pyridine nitrogen, respectively. Thus, the minimum solubility of 20 μmoles/liter at pH 6.5 is responsible for the poor solubility of its zwitter ion. Thus, after oral administration of I, the drug molecules dissolve easily in gastric juice but they precipitate rapidly in the intestinal fluid owing to inadequate pH of the fluid.

In conclusion, it was found in the present study that the bioavailability of I in terms of urinary recovery was incomplete and change with dose being subjected to a poor solubility of I in the intestinal fluid. Poor reabsorption of II–G in the intestine was also considered to be responsible for the incomplete bioavailability owing to the possible low partition coefficient of II–G. The permeation of I through the intestine is considered to permeate without metabolism and the metabolism is possibly performed after absorption in the liver. The pharmacokinetic analysis of I in the living body is considered to be complicated owing to its rapid metabolism and the metabolites are easily excreted in the intestine through a biliary excretion. It is required that a definitive assessment of the physiological availability of I in dog must await further study with a more comprehensive pharmacokinetic analytical method.

8) H. Nowak and G. Schorre, *Arzneim.-Forsch.*, **19**, 11 (1969).
9) Referred from a thesis by K. Miyake of the Master of Pharmacy degree of Osaka University.