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Tissue Distribution and Metabolism of Drugs. II.[1] Accumulation and Permeation of Drugs in the Rabbit Pancreas[2]

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Tissue distribution and penetration of drugs were measured in the rabbit pancreas cannulated into the pancreatic duct for the collection of pancreatic juice.

Drugs with high lipid solubility were found to be easily distributed within the pancreas. The lipid solubility of drugs appears to be the most important factors determining their distribution in the pancreas.

Small molecules were rapidly penetrated from blood to pancreatic juice if they are lipid soluble. Permeability of drugs through the pancreas was demonstrated to be dependent upon their molecular size and lipid solubility.

Increased penetration of drugs by the retrograde infusion of $HgCl_2$ solution through the pancreatic duct suggested that both lipid barrier and molecular sieve barrier would be complex multicellular system and exist independently surrounding the pancreatic duct.

Keywords——distribution of drugs; permeation of drugs; accumulation of drugs; transport barrier; pancreas; pancreatic juice; retrograde injection; mercuric chloride

Drug concentration in blood has been used as an index of drug efficiency and dose scheduling for therapeutics. However, some cardiac drugs, short acting barbiturate or antiarrhythmic drugs have rather narrow margins of safety. Their kinetic distribution in blood may not provide sufficient information on the adequacy of therapy. The drug level in organ or tissue can provide much better information than that in blood as to whether the therapy is optimal or not. Furthermore, knowledge of the drug distribution in blood, organs and tissues may be necessary for providing optimal treatment or protection from adverse reaction.

From these situation, the characteristic of drug distribution has received increasing attention in recent years. Especially, the therapeutic and toxic effect of drugs on endocrine organs have become an important area of the dosage regimen. To understand better the pharmacological activity of various drugs in the pancreas, it is necessary to clarify the pharmacokinetic parameters that govern the distribution of drugs in the pancreas. In 1935, Ingraham and his co-worker made an attempt to correlate the permeation characteristic of dyes in the pancreas based on their ionic potency.[4] Recently, Atkins, *et al.*,[5] Sawabu, *et al.*,[6] and Noda, *et al.*[7] have demonstrated in the area of diagnostics the nature of distribution of ^{75}Se-methionine and dimethadione (DMO) in a variety of systems. Dainko, *et al.*[8] and Takayama[9] have studied the distribution and disposition of antibiotics and sulfisoxazole in

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2) Part of this work was presented at 96th Annual Meeting of Pharmaceutical Society of Japan, Nagoya, April, 1976.
3) Location: *Kasumi 1-2-3, Hiroshima.*
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5) H.L. Atkins, W. Hauser, and J.F. Klopper, *Metabolism*, **20**, 1052 (1971).
6) N. Sawabu, S. Hirose, S. Nakazima, M. Yoneda, K. Nishimura, and J. Takeuchi, *Nippon Shokakibyo Gakkai Zasshi*, **72**, 25 (1975).
7) A. Noda, Y. Toda, T. Hayakawa, and S. Nakajima, *Digestive Diseases*, **18**, 498 (1973).
8) E.A. Dainko, H.A. Paul, A. Gabel, and E.J. Beattie, *Arch. Surg.*, **86**, 1050 (1963).
9) H. Takayama, *Jap. J. Antibiotics*, **26**, 427 (1973).

the pancreas. However, the factors which regulate the *in vivo* uptake and permeation of drugs by the pancreas have not been fully elucidated.

In the present paper, observation on the *in vivo* drug uptake by the pancreas and the secretion of drugs into the pancreatic juice are described.

Materials and Methods

Animals——Male New Zealand white rabbits weighing 2.0—3.0 kg were used. They were housed in constant environment rooms and allowed free access to water and food.

Experimental Procedure——The rabbits were anesthetized with pentobarbital (27 mg/kg, *i.v.*) and kept warm by heat from an electric bulb. Polyethylene tubing (outside diameter 1.1 mm and inside diameter 1.0 mm from Igarashi Ika Kogyo Co., Ltd. Tokyo) was cannulated into the femoral artery to collect blood samples In order to prevent clotting of blood samples, heparin sodium (200 U/kg, NOVO Inc. Denmark) was administered through the cannulated tubing. Collection of pancreatic juice was performed by cannulation of the pancreatic duct according to a minor modification of the procedure described by Matsunaga.[10] A 23-gauge needle was inserted through the intestinal wall at the opposite side of the pancreatic duct. After removal of the needle, polyethylene tubing, PE-50 (outside diameter 0.97 mm and inside diameter 0.58 mm from Cray-Adams, Division of Becton-Dickinson, N.J., USA) with a sharp tip was inserted into the same hole and cannulated into the pancreatic duct. This tubing was secured by careful ligation around the point of insertion. Secretin (2 CHR-U/kg/hr, Eisai Inc. Tokyo) was infused through the ear vein in order to maintain a stable flow rate of pancreatic juice. Thirty to 60 minutes after cannulation, a stable flow rate of pancreatic juice ranging from 20 to 40 μl/min/pancreas was achieved. Pancreatic juice was collected at 15-minute intervals under a small amount of paraffin oil. During the operation, hemorrhage was carefully prevented. The plasma concentrations of all drugs studied were established and maintained constant by a suitable combination of priming injections and continuous *i.v.* infusion. At the end of the animal experiment, blood was drained and the pancreas was removed for the estimation of its drug content. Plasma level of unbound drug was determined by an ultrafiltration technique.[11]

Retrograde Injection of $HgCl_2$ Solution——For modification of the surface barrier in the pancreatic duct, 0.1 ml of 0.01% $HgCl_2$ solution containing phenol red as an indicator was injected retrogradely into the pancreatic duct system through the cannula. The injected solution was left there for 1 min as was adopted in the modification of transport barrier of salivary gland[12] after which the occlusion was released. Collection of the pancreatic juice was begun after a color indicator, phenol red, introduced in $HgCl_2$ solution was cleared as indicated by the loss of red color to the secretion.

Materials——Sulfanilamide derivatives, isonicotinic acid derivatives, and other drugs used in this report are listed in Table I. All of the drugs were obtained from commercially available sources. All other chemicals were of analytical grade.

Preparation of Injectable Solutions——The drug solutions for priming injection and constant infusion were prepared as isotonic solutions, and their pH were adjusted at 7.40—7.45. For cases in general, drug concentrations of 50 or 150 mM were employed. However, concentrations of 15 and 8 mM respectively were used for sulfisomidine and sulfathiazole, because of their poor solubilities in water.

Analytical Methods——The concentrations of sulfanilamide derivatives and procainamide in plasma, plasma ultrafiltrate, pancreatic juice, and homogenate of pancreas were determined by the modified method of Bratton and Marshall[13] after deproteinization. Isonicotinic acid derivatives were analyzed by the method of Nielsh.[14] Determination of dimethadione (DMO) was made by the method of Waddle.[15]

Results and Discussions

Distribution of Drugs in the Pancreas

In order to determine the distribution characteristic of different drugs, drug uptake by the pancreas after the two hours of constant infusion was studied. Table I illustrated the concentration ratio of these drugs in the pancreas to the plasma concentration of unbound

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TABLE I. Distribution of Drugs in Pancreas

Drug	M.W.	P.C.[a]	Panc/Pf±SEM[b]
Procainamide	235.3	0.27	1.18±0.32 (4)
Sulfisomidine	278.3	0.16	1.00±0.14 (3)
Sulfathiazole	255.3	0.046	0.98±0.05 (3)
Sulfanilamide	172.2	0.026	0.95±0.12 (5)
Isonicotinamide	122.1	0.046	0.73±0.03 (5)
Dimethadione	129.1	0.022	0.72±0.07 (7)
Sulfisoxazole	267.3	0.024	0.58±0.04 (5)
Isonicotinic acid	123.1	0.00015	0.43±0.07 (7)
Sulfanilic acid	173.8	0.00001	0.25±0.02 (4)

a) Partition coefficient was measured between $CHCl_3$ and phosphate buffer (pH 7.4) at 37°.
b) Panc/Pf indicates the concentration ratio of drug in the pancreas to unbound drug in the plasma. These data were obtained two hours after the constant infusion and represent the mean ± standard error of mean (S.E.M.) with the number of animals in parentheses.

drugs which is expressed as the distribution ratio (Panc/Pf). The concentration of sulfisomidine, sulfathiazole, and procainamide in the pancreas attained almost same level as the plasma concentration, whereas sulfanilic acid, isonicotinic acid, and sulfisoxazole were accumulated at a lower distribution ratio. None of the drugs studied attained a distribution ratio greater than 1.2. These findings suggest that there would be no specific binding site for these drugs in the pancreas. The lipid solubilities are also listed in Table I as partition coefficients between chloroform and isotonic phosphate buffer (pH 7.4). The correlation coefficient calculated for distribution ratios and partition coefficients of all drugs tested was 0.90 and highly significant ($p<0.01$), while the distribution ratio of isonicotinic acid was slightly higher than expected on the basis of its partition coefficient. These data demonstrate that the rate-limiting factor for distribution of these drugs in the pancreas is the lipid solubility of the drugs at physiological pH. According to the generally accepted hypothesis, drugs with high lipid solubilities would be expected to easily penetrate the biological membranes. A comparison of distribution of the drugs tested suggested that drug penetration might be an important process for the drug distribution in the pancreas. Although special accumulation in the pancreas or pancreatic islet cells for some chemicals and peptide have been reported,[5,16] no special pattern of distribution was detected in this experiment.

TABLE II. Permeation of Drugs from Blood to Pancreatic Juice

Drug	J/Pf±SEM[a]	J/Panc±SEM[b]
Dimethadione	1.80±0.09 (7)	2.71±0.26 (6)
Isonicothinamide	0.92±0.02 (5)	1.26±0.03 (5)
Sulfanilamide	1.05±0.02 (8)	1.26±0.17 (4)
Isonicotinic acid	0.19±0.02 (7)	0.47±0.06 (6)
Sulfanilic acid	0.06±0.02 (6)	0.19±0.07 (4)
Sulfisoxazole	0.12±0.01 (11)	0.21±0.01 (5)
Sulfathiazole	0.17±0.03 (3)	0.18±0.04 (3)
Sulfisomidine	0.20±0.06 (5)	0.16±0.04 (3)
Procainamide	0.10±0.01 (4)	0.10±0.02 (4)

a) J/Pf indicates the concentration ratio of drug in the pancreatic juice to unbound drug in the plasma.
b) J/Panc indicates the concentration ratio of drug in the pancreatic juice to drug in the pancreas.
These data were obtained two hours after the constant infusion and represent the mean ± standard error of mean (S.E.M.) with the number of animals in parentheses.

16) H. Tjälve and E. Wilander, *Experientia*, **31**, 1061 (1975); G. Lanciault, J. Gilson, J.P. Accary, and S. Bonfils, *Proc. Soc. Exp. Biol. Med.*, **148**, 1081 (1975).

Drug Permeation from Blood to Pancreatic Juice

The plasma and pancreatic juice levels obtained following the administration of procainamide, sulfisoxazole, sulfanilamide, and DMO are presented in Figures 1 to 4 as a function of time. The concentrations of these drugs in plasma and pancreatic juice were almost identical after the 30 min infusion, indicating that a state of equilibrium was achieved at that time. The concentration ratios of pancreatic juice to unbound plasma concentration (J/Pf) and pancreatic juice to pancreatic concentration (J/Panc) of these drugs after the two hours of constant infusion are listed in Table II.

Procainamide was used as a model for basic drugs. As shown in Fig. 1 and Table II, the concentration of procainamide in pancreatic juice was low compared to the plasma unbound level and the content in the pancreas. One possible reason for this was considered to be the difference between the pH of plasma and pancreatic juice. The pH of the pancreatic juice sample ranged from 8.0 to 8.4 (8.26 ± 0.16) which is considerably different from plasma pH and the intracellular pH of pancreatic cells. Procainamide is a weak base with pK_a of 9.4. The unionized form of the drug is more lipid soluble than the cation and would be expected to diffuse more freely across the pancreatic barrier. The theoretical distribution ratio derived from the Henderson-Hasselbach equation is 0.11 to 0.26 which is somewhat higher than the observed ratio.

Acidic drugs were studied to determine if the amount excreted into the pancreatic juice is dependent upon the pH-partition hypothesis. The plasma and pancreatic juice levels obtained following the administration of sulfisoxazole are presented in Fig. 2. It was demonstrated that the concentration of sulfisoxazole in the pancreatic juice was extremely lower than the unbound level in the plasma. Almost the same results were obtained following the administration of sulfathiazole and sulfisomidine as shown in Table II. Although the theoretically calculated concentration ratios (J/Pf) of these drugs were greater than 2, experimentally determined concentration ratios were below 0.20. The low concentration ratios of these acidic drugs cannot be explained only on the basis of pH-partition hypothesis which can well account for the mechanism of drug secretion in the stomach[17] and mammary

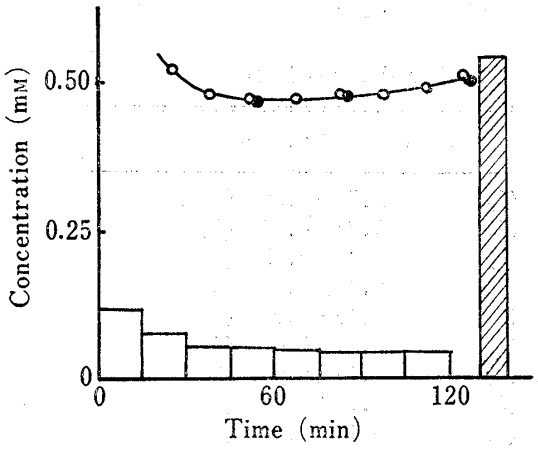

Fig. 1. Concentration Profile of Procainamide

—○—, drug concentration in plasma.
—●—, free drug concentration in plasma.
Open and hatched columns denote drug concentration in pancreatic juice and pancreas as stated in method.

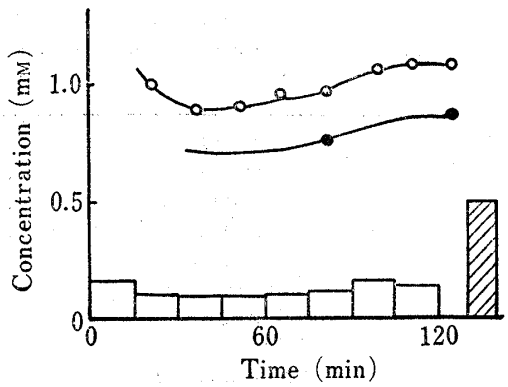

Fig. 2. Concentration Profile of Sulfisoxazole

—○—, drug concentration in plasma.
—●—, free drug concentration in plasma.
Open and hatched columns denote drug concentration in pancreatic juice and pancreas as stated in method.

17) P.A. Shore, B.B. Brodie, and C.A.M. Hogben, *J. Pharmacol. Exp. Ther.*, **119**, 361 (1957).

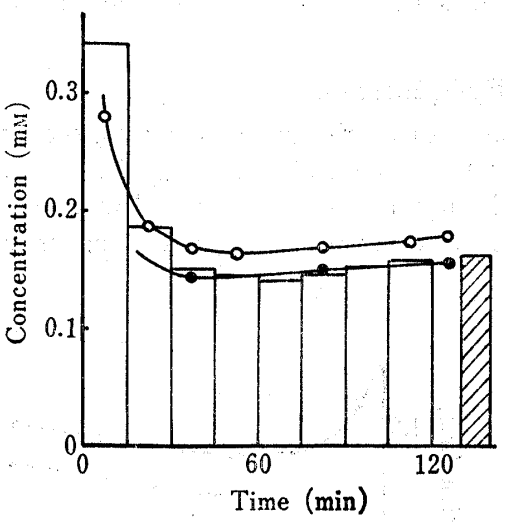

Fig. 3. Concentration Profile of Sulfanilamide

—○—, drug concentration in plasma.
—●—, free drug concentration in plasma.
Open and hatched columns denote drug concentration in pancreatic juice and pancreas respectively as stated in method.

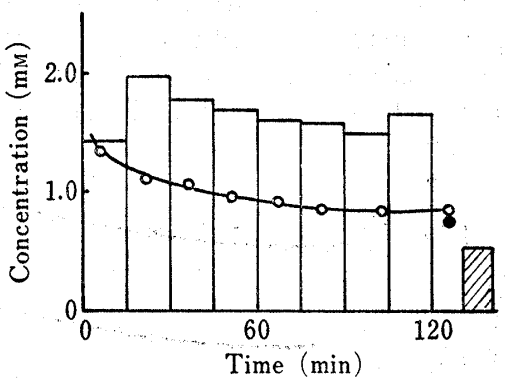

Fig. 4. Concentration Profile of Dimethadione

—○—, drug concentration in plasma.
—●—, free drug concentration in plasma.
Open and hatched columns denote drug concentration in pancreatic juice and pancreas as stated in method.

gland,[18] and thus must be due to several other factors; *e.g.*, the molecular size, the flow rate of pancreatic juice, etc.

As shown in Fig. 3 and Table II, the effect of molecular size on drug transport into the pancreatic duct was studied. It is evident that the pancreatic juice rapidly attained equilibrium with plasma sulfanilamide and isonicotinamide. The concentration ratios of drugs in the pancreatic juice to unbound drug in the plasma (J/Pf) were 1.05 for sulfanilamide and 0.92 for isonicotinamide. It may be assumed that the comparatively small molecular size of these drugs is the reason for their high permeability. In the case of sulfanilic acid and isonicotinic acid which have very low lipid solubilities and small molecular weights, the concentration ratios (J/Pf) were similar to those obtained for sulfisoxazole, sulfathiazole, and sulfisomidine. From these results, it appears that permeability of drugs across the transductular barrier of pancreas depends on both molecular size and lipid solubility, and also appears that large molecules such as sulfisomidine and sulfathiazole are hardly permeable though their lipid solubility resemble that of sulfanilamide and isonicotinamide.

In order to reinforce the impression derived from these data, DMO was examined for its permeability characteristic in the pancreas. Figure 4 represents the concentration profile in the pancreatic juice and plasma of DMO which does not bind with plasma protein. As expected, DMO was rapidly transported into the pancreatic duct and concentration of DMO in the pancreatic juice exceeded the plasma level considerably. Since DMO is an acidic drug with pK_a of 6.1, this finding is in accordance with the theoretical disposition profile of this drug when the undissociated form of DMO can cross the barrier freely. Drugs and chemicals are known to be transported across cell membranes by processes that include filtration through pores, simple diffusion through the lipoidal membrane, and specialized transport processes. The data reported here suggest that the permeability of the transductular barrier in the pancreas is largely dependent on both the molecular size and lipid solubility which correspond to filtration through the pores and simple diffusion through the membrane respectively. It is of further interest that both molecular size and lipid solubility are essential factors for drug transport from plasma to pancreatic juice, while the lipid solubility is the

18) G.E. Miller, N.C. Banerjee, and C.M. Stowe Jr., *J. Pharmacol. Exp. Ther.*, **157**, 245 (1967).

major factor for permeation of widely used drugs in the salivary gland,[19] mammary gland,[18] intestine,[20] and testis.[21]

Modification of Transductular Barrier by Retrograde $HgCl_2$ Injection

In order to gain further understanding of the transport barrier in the pancreas, the effect of partial modification of the barrier introduced by $HgCl_2$ was elucidated in Fig. 5 and Fig. 6. Sulfisoxazole was used as a model drug for large molecules and isonicotinic acid was selected as a model drug for lipid-insoluble compounds. Due to diversity in the path lengths from

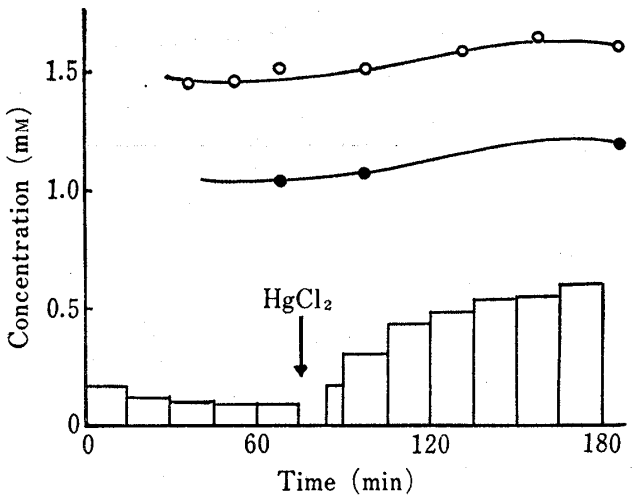

Fig. 5. Concentration Profile of Sulfisoxazole

—○—, drug concentration in plasma.
—●—, free drug concentration in plasma.
Histogram denotes drug concentration in pancreatic juice. $HgCl_2$ solution was injected retrogradely as stated in method.

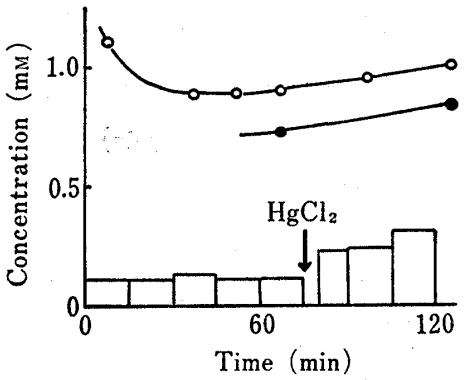

Fig. 6. Concentration Profile of Isonicotinic Acid

—○—, drug concentration in plasma.
—●—, free drug concentration in plasma.
Histogram denotes drug concentration in pancreatic juice. $HgCl_2$ was injected retrogradely as stated in method.

the acini through their ducts to the common duct outlet, the retrograde injection of the duct may be expected to affect a large percentage of the duct cells, some of the acini, and permeability barrier. The functional test of pancreatic secretion using the flow rate of pancreatic juice indicated that the effect of $HgCl_2$ solution was not so serious. The pH of pancreatic juice was also checked before and after the retrograde injection of $HgCl_2$, and no significant difference was observed ($8.02\pm0.27\rightarrow8.12\pm0.22$). It was noted that there was prominent increase of sulfisoxazole and isonicotinic acid concentrations in the pancreatic juice after retrograde $HgCl_2$ injection, though the flow rate and pH of pancreatic juice remained unchanged. These results indicate that both transport barriers for sulfisoxazole and isonicotinic acid were affected by the retrograde $HgCl_2$ injection, and it is anticipated that the transport barrier which is restrictive for large molecules and lipid-insoluble compounds may be complex multicellular system and be located independently near the surface of the pancreatic duct.

Our study reveals that drug disposition in the pancreas was regulated by certain passive transport characteristics. The drug disposition in many organs should always be taken into consideration for dose scheduling in therapeutics, and these data may give some information in providing guidance to effective and safe usage.

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20) L.S. Schanker, D.J. Tocco, B.B. Brodie, and C.A.M. Hogben, *J. Pharmacol. Exp. Ther.*, **128**, 81 (1958).
21) K. Okumura, I.P. Lee, and R.L. Dixon, *J. Pharmacol. Exp. Ther.*, **194**, 89 (1975).