[Chem. Pharm. Bull. 17(12)2475—2483(1969)] UDC 615.356.03 : 577.164.13

Studies on Fatty Acid Esters of Pyridoxine. VI.[1] Effects of Surface-active Agents on the Gastro-intestinal Absorption of Pyridoxine 3,4-Dipalmitate in Man[2]

Nobuyasu Mizuno, Akira Kamada, Noboru Yata and Masaru Aoki

Faculty of Pharmaceutical Sciences, Osaka University[3]

(Received March 19, 1969)

Pyridoxine 3,4-dipalmitate (PIN–DP), insoluble in water, was little absorbed in man following oral administration of suspension in water or aqueous Tween 80. But, the absorption increased markedly with a solubilized solution with Tween 80.

Previously, it was found that PIN–DP failed to permeate the intestinal tract unlike pyridoxine (PIN) which was the hydrolyzed product of ester in the tract.

Hydrolysis of PIN–DP solubilized with Tween 80 increased ten times with the addition of sodium taurocholate or glycocholate. But, no increase was observed with the addition of sodium lauryl sulfate or bis(2-ethylhexyl)sulfosuccinate. PIN permeated through the everted sac of rat intestine following the addition of sodium taurocholate and lipase to a solubilized Tween 80 solution of PIN–DP.

Vitamin B_6 was rapidly absorbed from the small intestine in man by a passive diffusion rather than by an active transport.[4] Seventy % of the metabolites of pyridoxine in the urine proved to be 4-pyridoxic acid (PiA).[5]

It was reported[1] that pyridoxine 3,4-dioctanoate (PIN-DK) and pyridoxine 5-octanoate were easily absorbed through the gastrointestinal tract in man like pyridoxine hydrochloride (PIN–HCl) following a single oral administration. But pyridoxine 3,4-dipalmitate (PIN–DP) had no influence on the urinary excretion of PiA following a single oral administration of PIN–DP powder with water. The poor absorption of PIN–DP in man was closely correlated with a poor hydrolysis by lipase and esterase extracted from mouse tissues. Thus, differences in absorption, excretion, and availability of pyridoxine esters following an oral administration were seemed to be responsible for these esters' stability against enzymatic hydrolysis in the gastrointestinal tract of man.

Unlike its poor absorption following an oral and a percutaneous[6] administration, PIN–DP was credited for pharmaceutical purposes with its easy synthesis and good stability in pharmaceutical preparations.

In general, absorption of drugs can be enhanced by the modification of water solubility as well as dispersibility in water.[7] To improve the absorption of poor water-soluble drugs, the followings are generally considered:

1) reduction of particle size,[8]

1) Part V: N. Mizuno, M. Aoki and A. Kamada, *Bitamin*, **38**, 129 (1968).
2) Part of this work was reported at the 18 th Annual Meeting of Kinki Branch, Pharmaceutical Society of Japan, Nov. 1968.
3) Location: *Toneyama, Toyonaka, Osaka.*
4) C.C. Booth and M.C. Brain, *J. Physiol.*, **164**, 284 (1962); M.C. Brain, J.S. Steware and C.C. Booth, *Strahentherapie*, Sonderbaede, **5**, 475 (1962).
5) J.C. Rabinowitz and E.E. Snell, *Proc. Soc. Exptl. Biol. Med.*, **70**, 235 (1949).
6) N. Mizuno, M. Aoki and A. Kamada, *Chem. Pharm. Bull.* (Tokyo), **17**, 669 (1969).
7) N. Mizuno, M. Aoki, A. Kamada and F. Hirota, *Yakuzaigaku*, **28**, 159 (1968).
8) K. Kakemi, T. Arita and S. Ohashi, *Yakugaku Zasshi*, **82**, 1468 (1962).

2) addition of auxiliary substances,[9]
3) solubilization with surface-active agents.[10]

Recently nonionic surface-active agents have been increasingly used as emulsifier, solubilizer and adjuvant in pharmaceutical preparations because of their low toxicity for the living body. Effects of surfactants on drug absorption have been variously reported such as increase, decrease and no change.[11] Much of the complexity with the effects might be due to the physicochemical nature of surfactants as well as their physiological property for living body.

In case of gastrointestinal absorption, attention should be given to the presence of surface-active substances contained primarily in the digestive fluid.

Presently, the effects of nonionic surface-active agents on the intestinal absorption of PIN-DP in man were studied. And the importance of surface-active substances in the bile for absorption was suggested.

Results and Discussion

Intestinal Absorption in Man

Solubilizing ability of nonionic surface-active agents for PIN-DP was examined for Tweens, Myrjs and sugar esters of fatty acids. In the present study, Tween 80 (Atlas Chemical Ind.) was used because of its good solubilizing ability and low toxicity in oral administration to man.

The absorption and availability of PIN-DP following an oral administration in man were studied by measuring the urinary excretion of PiA, one of principal metabolites of PIN[5,12] and its ester.[1] Kakemi, *et al.* reported that the urinary excretion of PiA was proportional to a single dose of PIN-HCl orally administered to man.[12] Thus, the absorption of PIN and its esters in man can be sufficiently evaluated by the determination of PiA in the urine.[5,12]

PIN-DP, 243.3 μmoles solubilized in 100 ml of 1% aqueous solution of Tween 80, was orally administered to man. The excreted amount of PiA in the urine was measured fluorophotometrically (Table I). The main metabolite of the compound was identified as PiA by thin-layer chromatography (TLC).[1]

Table I. Cumulative Urinary Excretion of 4-Pyridoxic Acid following an Oral Administration of 243.3 μmoles of PIN-DP solubilized in Aqueous Tween 80

Time (hours) / Subjects	2	4	6	8	10	12	14	24	48
	(μmole)								(μmole)
NM	21.7	52.1	60.9	65.7	69.1	72.2	74.5	84.8	105.3
	20.3	46.8	59.4	67.6	73.3	78.8	84.0	100.5	122.0
	19.2	47.5	61.8	71.8	78.5	82.6	86.6	101.1	122.3
Mean	20.4	34.8	60.7	68.4	73.6	77.9	81.7	95.5	116.5
KM	8.2	21.8	30.2	36.6	41.7	45.9	49.4	69.8	93.4
	7.8	27.9	41.1	51.1	59.9	67.9	72.4	93.6	111.6
Mean	8.0	24.9	35.7	43.9	50.8	56.9	60.9	81.7	102.5

The urinary excretion of PiA following an oral administration of the same amount of PIN-DP (particles were passed through a 200-mesh sieve) suspended into 100 ml of 1% aqueous solution of Tween 80 was measured (Table II).

9) G. Levy and R.H. Reuning, *J. Pharm. Sci.*, **56**, 843 (1967).
10) G. Levy, K.E. Miller and R.H. Reuning, *J. Pharm. Sci.*, **55**, 394 (1966).
11) I. Utsumi, M. Samezima and I. Sugimoto, *Yakkyoku*, **16**, 987 (1965).
12) K. Kakemi, J. Kozatani and T. Tsuchiya, *Yakuzaigaku*, **27**, 210 (1967).

Table II. Cumulative Urinary Excretion of 4-Pyridoxic Acid following an Oral Administration of 243.3 μmoles of PIN-DP suspended in Aqueous Tween 80

Time (hours) / Subjects	2	4	6	8	10	12	14	24	48 (μmole)
	(μmole)								
NM	1.0	2.4	5.5	8.5	10.6	12.3	13.9	26.1	37.2
	0.9	3.6	6.2	8.6	10.1	11.0	12.8	21.4	29.8
	0.4	0.9	4.8	7.4	8.8	10.0	11.5	15.8	25.1
Mean	0.8	2.3	5.5	8.2	9.8	11.1	12.7	21.1	30.7
KM	0.2	0.6	1.0	2.1	3.2	6.1	6.4	15.9	24.0
	0.2	0.5	1.0	1.5	1.8	2.2	2.9	7.2	13.5
Mean	0.2	0.6	1.0	1.8	2.5	4.2	4.7	11.6	18.8

Table III. Cumulative Amounts of 4-Pyridoxic Acid in the Urine during 48 Hours following an Oral Administration of 243.3 μmoles of PIN-DP solubilized or suspended in Aqueous Tween 80

Condition	Mean S.D. (μmole/48 hours)	Availability
PIN-DP solubilized with aqueous Tween 80	110.9±10.9	0.791
PIN-DP suspended in aqueous Tween 80	25.9± 7.8	0.185
PIN-DP suspended in water	22.5± 8.5	0.160
PIN-HCl dissolved in aqueous Tween 80	138.3± 7.2	0.986
PIN-HCl dissolved in water	140.2± 6.8	1.0

For the sake of comparison, the mean values of urinary excretion of PiA for the five preparations during 48 hr following a single oral administration of solubilized and suspended PIN–DP in aqueous Tween 80 solution were presented as well as those of aqueous suspension of PIN–DP, and aqueous and Tween 80 solutions of PIN–HCl (Table III).

Little influence of Tween 80 on the absorption of PIN–HCl was observed. The availability of PIN–HCl for oral administration is assumed as around unity because of its rapid absorption and excretion in man.[1] Thus, the ratio of PIN–DP against PIN–HCl in terms of PiA excreted in the urine during 48 hr may represent the relative availability for suspended and solubilized preparations. Availability of PIN–DP for suspensions in water and aqueous Tween 80 solution was poor, but it was markedly enhanced by solubilization with Tween 80. Little influence of Tween 80 on the

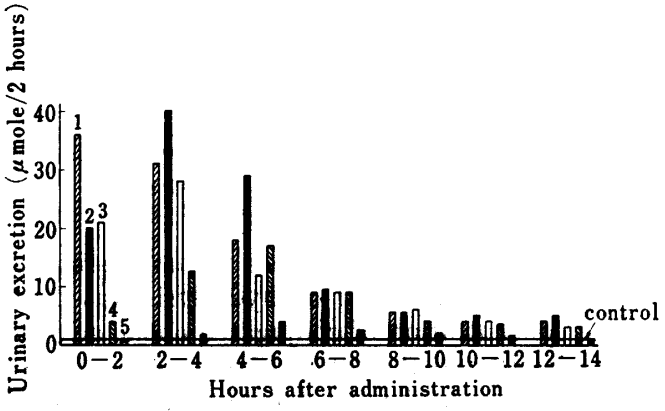

Fig. 1. Urinary Excretion of 4-Pyridoxic Acid following an Oral Administration of 243.3 μmoles of PIN-DP solubilized and suspended in Aqueous Tween 80 as well as the Same Amounts of PIN-DP, PIN-DL and PIN-DK suspended in Water and Aqueous Solution of PIN-HCl

1 : PIN–HCl dissolved in water
2 : PIN–DK suspended in water
3 : PIN–DP solubilized in aqueous Tween 80
4 : PIN–DL suspended in water
5 : PIN–DP suspended in water or aqueous Tween 80

absorption and availability was observed for aqueous suspensions. It means that the rate of solubilization of PIN–DP in aqueous Tween 80 solution is small and little is solubilized into micelles of Tween 80 in the gastrointestinal tract.

Mean chronological excretion rates of PiA (μmole/2 hr) in Table I and II for one of two subjects were presented in Fig. 1 as well as the rates for aqueous suspensions of PIN–DK and PIN–DL (pyridoxine 3,4-dilaurate) and aqueous solution of PIN–HCl.[1] The maximum excretion rate was observed 4—6 hr after administration for suspensions of PIN–DL and –DP. But, it appeared 2—4 hr after administration for a solubilized preparation of PIN–DP. It suggests a rapid absorption from a solubilized preparation.

From the difference in absorption of solubilized and suspended preparations, it is considered that the effect of Tween 80 on the intestinal absorption of water–insoluble PIN–DP is not referable to surface–activity but the solubilizing ability. Therefore a pre–solubilization is required for the oral administration. The poor absorptivity of PIN–DL can be also improved by being solubilized into Tween 80 before the administration.

Effects of Sodium Taurocholate and Sodium Glycocholate on the Hydrolysis of PIN-DP by Lipase or Homogenate of Rat Intestine

Previously, it was reported[7] that PIN–DK was hydrolyzed easily, PIN–DL slightly and PIN–DP little by lipase or homogenates of animal tissues. The difficulty of hydrolysis of esters by enzymes *in vitro* was found to be closely related with their poor absorptivity through the intestinal tract and low excretion in the urine.[1] Only PIN was responsible for the intestinal absorption *via* hydrolysis of esters by enzymes in the intestinal fluid.[13] The enhanced absorption of PIN–DP in a solubilized preparation is to be considered on the basis of hydrolysis by esterase in the intestinal fluid.

Enzymatic hydrolysis of PIN–DP *in vitro* was little influenced by the solubilized and suspended aqueous solutions of Tween 80 (Table IV).

Table IV. Hydrolysis of PIN-DP by 9 mg/ml Lipase or 90 mg/ml Homogenates of Animal Intestine at 37° for 1 hour

Enzymes	Tween 80 or propylene glycol	Hydrolysis (%)
Lipase		2.0
Mouse intestine	solubilized with aqueous Tween 80	2.4
Rat intestine		2.1
Lipase	suspended in 9% propylene	3.0
Mouse intestine	glycol	2.2

To explain a poor hydrolysis *in vitro* against a good absorption *in vivo*, the effect of bile salts in the digestive fluid on hydrolysis was studied.

The concentration of conjugated cholic acid salts in the human intestinal fluid is around 0.01 M or less[14] and the ratio of conjugates with glycine to taurine is 3:1.[15] Thus, the concentration of salts of taurocholate and glycocholate is assumed to be 0.0025 M and 0.0075 M, respectively.

The addition of sodium salt of taurocholate (Na–T), glycocholate (Na–G) or cholate (Na–C) markedly increased the hydrolysis of PIN–DP solubilized in aqueous Tween 80 solution with lipase (Tables V and VI). In the present experiments, PIN–DP was solubilized into a Krebs–Ringer's solution of Tween 80 at pH 7.4. The activity of Na–G for the enhancement of hydrolysis was considered to be stronger than Na–T. It was interesting that hydrolysis was

13) N. Mizuno, M. Aoki, A. Kamada and S. Sumimoto, *Bitamin*, **38**, 125 (1968).
14) B. Borgström, "Lipid Metabolism," Wiley, New York, 1960.
15) T. Naito and K. Kobayagawa, *Saishin Igaku*, **20**, 3078 (1965).

Table V. Effect of Sodium Taurocholate on the Hydrolysis (%) of PIN-DP, solubilized with Aqueous Tween 80, by Lipase or Homogenate of Mouse Intestine

Enzymes (mg/ml)		Incubation time (hours)	Sodium taurocholate (μmole/ml)	Hydrolysis (%) Mean S.D.
Lipase	0.9	1	9.0	10.7±0.90
	0.9	3	9.0	16.6±0.64
	9.0	1	9.0	26.3±0.70
	9.0	3	0.09	6.2±0.55
	9.0	3	9.0	43.1±0.22
Lipase	9.0		—	3.9±0.07
Mouse intestine			9.0	2.9±0.21
	90	3	—	2.4±0.20
	—		9.0	0
	—		—	0

— denotes the absence of lipase or sodium taurocholate

Table VI. Effect of Sodium Glycocholate or Sodium Cholate on the Hydrolysis (%) of PIN-DP, solubilized with Aqueous Tween 80, by Lipase or Mouse Intestine at 37°

Enzymes (mg/ml)		Incubation time (hours)	Sodium glycocholate or sodium cholate (μmole/ml)		Hydrolysis (%) Mean S.D.
Lipase	9	1	sodium glycocholate	9	28.9±0.53
Lipase	9	2		9	39.5±0.41
Lipase	0.9	3		9	27.9±0.73
Lipase	9	3		0.9	14.7±0.22
Lipase	9	3		9	48.4±0.40
Lipase	9	3		—	3.9±0.07
Mouse intestine	90	3		9	3.3±0.23
Lipase	9	3	sodium cholate	9	62.0±0.81

—) denotes the absence of sodium glycocholate

not observed in suspensions of Tween 80 solution even with the addition of Na–T, Na–G or Na–C. Little influence of the addition of Na–T, Na–G or Na–C was observed on the hydrolysis of solubilized PIN–DP with the homogenate of the mouse intestine. The poor influence of bile salts was considered to be attributable to an insufficient amount of enzyme and/or low enzymatic activity of the mouse homogenate. The solubilizing ability of aqueous Na–T, Na–G and Na–C for PIN–DP was poor, so that no influence was observed on the hydrolysis of PIN–DP suspensions in the bile salt solution either with lipase or homogenate of the mouse intestine.

The effect of the concentration of Tween 80 on hydrolysis was studied (Table VII). Six mg of Tween 80 was a minimum amount to solubilize 2.37 μmoles of PIN–DP in 11 ml of buffer. Hydrolysis following a 3 hr incubation decreased with an increase of Tween 80 in the presence of the same amount of Na–T (9 μmoles/ml). An optimum concentration of Tween 80 is required for solubilization and hydrolysis.

Effect of Anionic Surface-active Agents on the Hydrolysis of PIN-DP solubilized with Tween 80

Na–T and Na–G, anionic surface–active materials, are primarily contained in the intestinal fluid. Thus, low toxic anionic surfactants, sodium lauryl sulfate (Na–L) and sodium

Table VII. Effect of Tween 80 Concentration on the Hydrolysis (%) of solubilized PIN-DP in the Presence of 9 mg/ml Lipase and 9 μmoles/ml Sodium Taurocholate at 37° for 3 Hours

Tween 80 (%)	Hydrolysis (%) Mean S.D.
0.054	43.1±0.22
0.270	37.2±0.10
0.540	28.3±0.12

Table VIII. Effect of Anionic Surface-Active Agents on the Hydrolysis of PIN-DP, solubilized with Aqueous Tween 80, by 9 mg/ml Lipase at 37° for 3 Hours

Surface-active agents (9 μmoles/ml)	Hydrolysis (%) Mean S.D.
Sodium lauryl sulfate	3.0±0.20
Aerosol OT	2.9±0.18

bis(2-ethylhexyl)sulfosuccinate (Aerosol OT), were used instead of cholates. No enhancement in hydrolysis was observed (Table VIII).

It is considered that the enhancement in hydrolysis following the addition of Na-T or Na-G is a specific phenomenon for primarily existent cholates. Such specific enhancement of hydrolysis was left unexplained now.

Hydrolysis of PIN-DP in Solubilized Solution with BC 30

Tween 80 has an ester linkage between polyhydric alcohol and oleic acid. Thus, either lipase or homogenate of animal tissues can hydrolyze Tween 80 as well as PIN-DP solubilized in the surfactant solution. To avoid possible influence of the hydrolysis of surfactant, employed to solubilize PIN-DP, poly (oxyethylene) (30 mole) cetyl ether (BC 30) was used (Table IX). BC 30 was a good solubilizer for PIN-DP like Tween 80.

Table IX Effect of 9 μmoles/ml Sodium Taurocholate on the Hydrolysis (%) of PIN-DP, solubilized with Aqueous BC 30, by 9 mg/ml Lipase at 37°

Sodium taurocholate	Incubation time (hours)	Hydrolysis (%) Mean S.D.
+	1	35.8±1.12
	2	43.7±0.42
	3	49.5±0.19
−	3	4.2±0.18

+ and − denote the presence and the absence of sodium taurocholate, respectively.

A marked enhancement of hydrolysis was observed with BC 30 and Na-T, but not with BC 30 alone.

Table X. Effect of 9 μmoles/ml Sodium Taurocholate on the Hydrolysis (%) of PIN-DP, solubilized with 0.054% Sodium Lauryl Sulfate,by 9 mg/ml Lipase at 37° for 3 Hours

Lipase	Sodium taurocholate	Hydrolysis (%) Mean S.D.
−	+	0 —
+	−	8.7±0.54
+	+	58.4±0.83

+ and − denote the presence and the absence of sodium taurocholate, respectively.

It may be concluded that hydrolysis of Tween 80 little influences the hydrolysis of PIN–DP and that the hydrolysis itself is little influenced by the chemical structure of nonionic surfactants. The primary requirement for the hydrolysis of PIN–DP is that PIN–DP is solubilized with nonionic surfactants in bile salt solution. Na–L was also found effective for the enhancement of hydrolysis like Tween 80 and BC 30 (Table X).

Thus, solubilization of PIN–DP with surfactants in the presence of bile salts is important to enhance the hydrolysis as well as to increase absorption and availability.

A similar experiment of hydrolysis was made with homogenate of the rabbit pancreas (Table XI). A similar increase in hydrolysis was observed with the addition of Na–T or rabbit bile juice into a solubilized solution with Tween 80.

Table XI. Effect of Bile of Rabbit or Sodium Taurocholate on the Hydrolysis (%) of PIN–DP, solubilized with Aqueous Tween 80, by 180 mg/ml Homogenate of Rabbit Pancreas or 9 mg/ml Lipase at 37° for 3 Hours

Enzymes	Bile or sodium taurocholate	Hydrolysis (%) Mean S.D.
Pancreas	—	3.0±0.22
	0.9 bile	15.0±2.26
	sodium taurocholate (9 μmoles/ml)	13.6±2.14
Lipase	0.9 bile	41.4±3.22
	1.8 bile	84.8±4.71

Permeation of PIN-DP through Everted Sac of the Rat Intestine

It was reported[13] that PIN–DK in aqueous solution of propylene glycol was completely hydrolyzed within 30 min on the mucosal side of the everted sac of the rat intestine, but only 24% on the serosal side. And the resulted PIN permeated through the rat intestine. But PIN–DP was not hydrolyzed on either side of the intestine, and no permeation of PIN–DP or PIN was observed.

Presently, a study was made of permeation through the rat intestine of PIN–DP solubilized with Tween 80 or BC 30 with or without Na–T and Na–G (Table XII).

Table XII. Permeation of PIN–DP, solubilized with Nonionic Surface–Active Agents, through the Everted Sac of Rat Intestine at 37°for 1 Hour

Additives	Percent of PIN in muucosal side	Serosal side (μmole/ml) PIN Mean S.D.	PIN–DP	Surface–active agents used to solubilize PIN–DP
Control	0	0 —	0	Tween 80
Lipase	2.1	0 —		
Lipase+Na taurocholate	12.5	0.011±0.001		
Lipase+Na glycocholate	11.8	0.010±0.001		
Control	0	0 —	0	BC 30
Lipase	2.0	0 —		
Lipase+Na taurocholate	13.3	0.012±0.001		

The addition of lipase to solubilized PIN–DP resulted in a weak hydrolysis but no penetration of PIN or PIN–DP was observed. Bile salts markedly enhanced hydrolysis resulting in permeation of a considerable amount of PIN and little of PIN–DP.

The material permeated in the serosal side of an everted sac was identified as PIN by thin-layer chromatography. If any part of the rat intestine was taken, there was little influence

on hydrolysis and permeation. Thus, it is concluded that an intestinal absorption of PIN–DP proceeds after the hydrolysis of the ester. Poor hydrolysis with homogenates of the animal intestine in Table IV seems to be due to an insufficient amount of esterase and/or its low activity for hydrolysis. Here the amount is important because of a marked hydrolysis observed with the addition of rabbit pancreas (Table XI).

Effect of Sodium Taurocholate and Sodium Glycocholate on the Partition of PIN-DP between Chloroform and Krebs-Ringer's Solution of Tween 80 or BC 30

Changes of hydrolysis with the addition of bile salts are interesting for the study of biopharmaceutical and physicopharmaceutical problems. It seems to be due to the change of structure of micelles and distribution of the drug in micelles as well as the change of the enzymatic activity in the presence of bile salts.

As a preliminary study, PIN–DP solubilized with Tween 80 in Krebs–Ringer's solution was extracted with $CHCl_3$. PIN–DP was completely extracted with $CHCl_3$. The addition of Na–T or Na–G resulted in a difficult extraction. Extraction of PIN–DP dissolved in $CHCl_3$ with a Krebs–Ringer's solution of Tween 80 and Na–T was unsuccessful. Thus, it may be considered that the addition of bile salts to a solubilized solution results in change in the distribution of PIN–DP molecules in micelles. Extraction of the drug solubilized in micelles with organic solvents seems to be carried out by partitioning drug molecules dissolved in water phase into organic solvent phase and/or by directly interacting micelles with organic solvent. Here, becaues of the insolubility of PIN–DP in water, the later extracting process may be more likely for the extraction of PIN–DP solubilized in aqueous Tween 80 solution with $CHCl_3$. However, the addition of Na–T or Na–G to a solubilized solution results in inhibition of a direct interaction between micelles and organic solvent by some mechanism.

The addition of anionic surfactants to a solubilized solution likewise resulted in non-extractability. Thus, an enhanced hydrolysis with the addition of bile salts is considered to be an increased interaction of PIN–DP solubilized in surfactant micelles with enzyme molecules and/or increased the enzymatic activity.

Conclusion

Generally, drugs solubilized into surfactant micelles or bound to polymers, are not considered to permeate freely through the gastrointestinal tract.[16] Since PIN–DP is insoluble in water, its molecules in a solubilized solution are mostly bound to surfactant micelles. Therefore, PIN–DP solubilized with a surfactant does not seem to be satisfactorily absorbed following an oral administration. But, the present study of oral administration of solubilized PIN–DP revealed a marked absorption and availability. In the previous[13] and present studies of permeation through an everted sac of the animal intestine, it was found that PIN, which was a product of hydrolysis in the intestinal tract, penetrated through the intestinal membrane. PIN–DP suspended in water or aqueous surfactant solution, or solubilized with a surfactant showed little hydrolysis even in the presence of lipase or homogenate of rat intestine. But, it showed a markedly increased hydrolysis with the addition of bile salts to solubilized solution. Bile salts have been recognized as an important factor as the absorption of fats.[17]

The present study also advocates the importance of bile salts for the absorption of drugs with ester linkage in molecules. A similar enhancement of absorption with the addition of bile salts to solubilized drug solution can be expected for the esters of chloramphenicol, erythromycin and vitamin C.

16) H. Yamada and R. Yamamoto, *Chem. Pharm. Bull.* (Tokyo), **13**, 1279 (1965); G. Levy, K.E. Miller and R.H. Reuning, *J. Pharm. Sci.*, **55**, 394 (1966).

17) C.A. Ross, A.C. Frazer, J.M. French, J.W. Cerrard, H. G. Sammons and J.M. Smellie, *Lancet*, **1**, 1087 (1955); T.H. Wilson "Intestinal Absorption," W.B. Saunders Co., London, 1962.

Experimental

Materials——Materials were used without further purification. Pyridoxine 3,4-dipalmitate, mol. wt. 646.01 and mp 88—89°, and BC 30 were obtained through the courtesy of the Nikko Chemicals Co., Ltd.

Oral Administration in Man——PIN–DP, 243.3 μmoles (157 mg) suspended or solubilized into 100 ml of 1% aqueous solution of Tween 80, was orally administered to two normal male subjects. The subjects were starved for 10 hr before the administration. The starvation was continued for another 5 hr. But, water was given freely during the experiment. Urine was collected for 14 hr with 2 hr intervals. The collection was continued for 48 hr.

Analysis of PiA in the Urine——PiA was measured fluorophotometrically following Møller's method[18] with a Shimadzu–Kotaki Model–UM ultramicro fluorophotometer.

Preparation of Solubilized Stock Solutions and Suspensions——Solubilized Stock Solutions: a) Intestinal absorption in Man: PIN–DP, 243.3 μmoles, was dissolved with 1 g of Tween 80 on a boiling water bath. A sufficient amount of water was added slowly under stirring in a 100 ml measuring flask.

b) Permeation through Everted Sac and Hydrolysis with Lipase: Six tenths g of Tween 80 or BC 30 and 237 μmoles of PIN–DP were mixed on a boiling water bath. Water was added under stirring to make 100 ml of solubilized solution.

Suspensions: Suspensions were usually prepared by suspending PIN–DP powder, passed through a 200 mesh sieve, in water or surfactant solution.

When a solubilized stock solution was diluted ten times with Krebs–Ringer's solution, kept at 37° for 1 hr in a water bath and filtered with filter paper, PIN–DP was proved to be still solublized 48% in the filtrate. But when PIN–DP was suspended in water or surfactant solution, no PIN–DP was found in the filtrate.

Hydrolysis with Lipase or Homogenate of Rat Intestine——One ml of the solubilized solution (b) of PIN–DP, 2.37 μmoles, 9 ml of a Krebs–Ringer's solution of pH 7.4 and 1 ml of one of stock solutions of Na–T or Na–G, dissolved in water at concentrations of 0.1, 0.01 and 0.001M were put in a 50 ml Erlenmeyer flask. After keeping the solution at 37° for 15 min, lipase, 10—100 mg, or homogenate of rat intestine, 1000 mg was added. The mixture was kept at 37° for 1—3 hr under shaking. Then, one ml of 10% aqueous solution of trichloroacetic acid was added to stop the reaction and remove protein. The concentration of PIN was measured colorimetrically with 2,6-dibromoquinone chlorimide.

Permeation through Everted Sac of Rat Intestine——The method with apparatus following Crane and Wilson[19] was used as reported in the previous paper.[13] The composition of solutions employed was as follows:

a) Mucosal Side	
Solubilized stock solution of PIN–DP (2.37 μmoles)	8 ml
0.1M aqueous solution of Na–T	8 ml
Krebs–Ringer's solution (pH 7.4) with 0.3% glucose	64 ml
Lipase	800 mg
b) Serosal Side	
Krebs–Ringer's solution (pH 7.4) with 0.3% glucose	2 ml

Hydrolysis was carried out at 37° for 1 hr under bubbling O_2 into solution of the mucosal side.

Partition of PIN-DP between Chloroform and Water——Eleven ml of a solubilized solution, which was used in the experiment of hydrolysis with lipase or homogenate of intestine, was shaken with 10 ml of $CHCl_3$ at 37° for 3 hr. The concentration of PIN–DP in the two phases was measured colorimetrically following hydrolysis with 1N NaOH. In case of partition without Na–T, 1 ml of water was added to the solubilized solution instead of aqueous Na–T solution.

18) P. Møller, *Acta Chem. Scand.*, **5**, 1418 (1951).
19) R.K. Crane and T.H. Wilson, *J. Appl. Physiol.*, **12**, 145 (1958).