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Solvolyses of Polyhydric Alcohol Esters. I. Stability of *meso*-Inositol Hexanicotinate in Acidic Medium

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The quantitative procedure of nicotinic acid liberated from *meso*-inositol hexanicotinate (IHN) and several nicotinic acid esters of polyhydric alcohols and the stability in acidic solution were investigated to predict the gastric stability for a possible explanation of their absorption from the gastrointestinal tract. Nicotinic acid formed by the degradation could be satisfactorily separated from the other degraded products by the ion-exchange chromatography. The results were in agreement with those of the titrimetry. The thin-layer chromatography on the degradation of IHN showed that IHN was solvolyzed to many intermediates which then successively decomposed to further degraded products. The initial solvolytic rate of IHN in 0.1N HCl and at 37.0° was faster than about ten times those of the other nicotinic acid esters used. The observed rate of the degradation of IHN was first order with respect to IHN concentration at a constant temperature and hydrogen ion concentrations by the thin-layer chromatographic technique. The variation of the rate constant was minor in the range of 0.04 to 1.0N HCl and at 37.0°. The dissolution of IHN from tablets, the enzymatic stability and the solubility of IHN were also discussed.

The attempt of conversion of water-soluble compounds, such as riboflavin and ascorbic acid, to fat-soluble derivatives by esterification of their hydrophilic groups have recently been made for purpose to sustain the *in vivo* drug level and/or to enhance the *in vitro* stability.[2] *meso*-Inositol hexanicotinate (IHN), one of these derivatives, is an ester of *meso*-inositol and nicotinic acid which are a lipotropic drug and a peripheral vasodilator drug, respectively. The use of nicotinic acid for vasodilating effect is limited by its short duration of action and occurrence of side effects.[3] IHN was found therapeutically to have a considerable advantage. It was possible to give a large dose of nicotinic acid as IHN without significant side effects and the vasodilating effect was prolonged. It was assumed that IHN had to be metabolized in order to bring about its effect and the action of IHN resulted from slow liberation of nicotinic acid.[4]

IHN is hardly soluble in water and alkaline solution, but dissolves in acidic solution. It is, therefore, postulated that most of IHN is absorbed as an ester in the stomach, and splits up in the blood probably by the pseudocholinesterase.[4] There have been several discussions[5-8] in the literature concerning the extent of absorption of IHN from the gastrointestinal tract. Vincke[5] reported that no increase of nicotinic acid level in the serum was found in humans after oral administration of 200—400 mg IHN. Harthon, *et al.*[6] claimed that the increase of nicotinic acid level in the whole blood and N-methylnicotinamide excreted in the urine were statistically shown in humans by oral dose 400 mg and 200 mg IHN, respectively. It

1) Location: *Hongo, Bunkyo-ku, Tokyo.*
2) K. Yagi, *Vitamins* (Kyoto), **33**, 465 (1966); H. Tanaka and R. Yamamoto, *Yakugaku Zasshi*, **86**, 376 (1966).
3) S.H. Lumish, C. Blyn, and T.H. Nodine, *Current Therap. Res.*, **4**, 243 (1962).
4) T. Lindqvist, *Svenska Lakartidn.*, **55**, 1 (1958).
5) E. Vincke, *Arzneimittel-Forsch.*, **13**, 734 (1963).
6) J.G.L. Harthon, K.J.E. Sigroth, and R.A. Sjobom, *Arzneimittel-Forsch.*, **14**, 126 (1964).

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was observed, further, that 70% of dose of IHN orally administered in 10 hospitalized patients was absorbed on the average by an analysis of the faeces, regardless of whether the drug was given with or between meals.[7] However, Tsuji, *et al.*[8] mentioned that no substantial evidence of absorption of IHN from the gastrointestinal tract was shown by analyses of nicotinic acid and N-methylnicotinamide in human and rat urine.

This paper reports on the quantitative determination of nicotinic acid liberated from IHN by an ion-exchange chromatography and on the formation rates of nicotinic acid from IHN and several nicotinic acid esters of polyhydric alcohols in acidic solutions. The purpose of this investigation was to obtain one of the data elucidating the process of the absorption of IHN from the gastrointestinal tract.

Experimental

Material——*meso*-Inositol hexanicotinate (IHN) was supplied by the Yoshitomi Pharmaceutical Industries, Ltd. *Anal.* Calcd. for $C_{42}H_{30}O_{12}N_6$: C, 62.21; H, 3.73; N, 10.37. Found: C, 62.03; H, 3.88; N, 10.39. Glycol dinicotinate, glycerol trinicotinate, erythritol tetranicotinate, pentaerythritol tetranicotinate and mannitol hexanicotinate were prepared by Method A of Badgett and Woodward.[9] The reaction products obtained were recrystallized from 40% ethanol except for glycerol trinicotinate (from isopropyl alcohol) and mannitol hexanicotinate (from acetone and petrolatum ether). Glycol dinicotinate, mp 126—127°. *Anal.* Calcd. for $C_{14}H_{12}O_4N_2$: C, 61.76; H, 4.44; N, 10.29. Found: C, 62.12; H, 4.63; N, 10.49. Glycerol trinicotinate, mp 88—89°. *Anal.* Calcd. for $C_{21}H_{18}O_6N_3$: C, 61.91; H, 4.20; N, 10.32. Found: C, 62.15; H, 4.18; N, 10.40. Erythritol tetranicotinate, mp 158—159°. *Anal.* Calcd. for $C_{28}H_{22}O_8N_4$: C, 61.99; H, 4.09; N, 10.33. Found: C, 61.75; H, 4.15; N, 10.08. Pentaerythritol tetranicotinate, mp 162—163°. *Anal.* Calcd. for $C_{29}H_{24}O_8N_4$: C, 62.59; H, 4.35; N, 10.07. Found: C, 62.82; H, 4.57; N, 10.26. Mannitol hexanicotinate, mp 232—233°. *Anal.* Calcd. for $C_{42}H_{30}O_{12}N_6$: C, 62.07; H, 3.97; N, 10.34. Found: C, 61.97; H, 4.07; N, 10.38. A buffered ammonia reagent for colorimetry of nicotinic acid was prepared by adding 6.7 ml of concentrated NH_4OH (28%) to an aqueous solution in which 87.0 g of K_2HPO_4 and 107.0 g of NH_4Cl were previously dissolved, and by making to 1000 ml with distilled water. Pepsin (1:5000) and pancreatin (N.F.) were obtained from Mikuni Kagaku Co. and Difco Laboratories, respectively.

Preparation of Column——The weak anionic exchange resin, Amberlite CG-4B (Type I) was used. Fifty grams of the resin was purified by soaking in distilled water, and washing with 500 ml of 0.5N HCl and subsequent washing with 500 ml of 0.5N NaOH in a column after removal of the acidic solution with distilled water. The resin was converted from its OH-form to its Cl-form again with an excess of 0.5N HCl. This product was washed with distilled water, and poured into a chromatographic tube making a column of 9×40 mm. Five milliliters of 1M acetate buffer solution (pH 4.9) was passed through the resin prior to addition of a sample solution.

Ion-Exchange Procedure——Five milliliters of a sample solution of degraded IHN or degraded nicotinic acid esters of polyhydric alcohols was adjusted to pH 2.0—2.3 by addition of 5 ml of NaOH solution in an appropriate concentration, and then the mixture was shaken with an equal volume of chloroform in a glass-stoppered tube for 10 min to remove slightly soluble compounds at pH 4.9, and centrifuged for 5 min. Five milliliters of the aqueous layer and 1 ml of 1M acetate buffer solution (pH 4.9) were pipetted into a reservoir connected to the column, and passed slowly through the column at a rate of 0.7 ml/min. The partially solvolyzed IHN or the partially solvolyzed nicotinic acid esters of polyhydric alcohols were eluted with distilled water alone into a 100-ml volumetric flask at a rate of 1.4 ml/min. The nicotinic acid absorbed to the resin was eluted with 0.3N HCl, and exact 50 ml effluent was collected in a 50-ml volumetric flask at a rate of 1.4 ml/min.

Colorimetry——The colorimetric method used for the determination of nicotinic acid liberated is based on the quantitative reaction of nicotinic acid with BrCN in the presence of ammonia.[10] Five milliliters of 10% BrCN was added to a mixture of 1 ml of the nicotinic acid-effluent, 1 ml of 0.3N NaOH and 3 ml of the buffered ammonia reagent. The maximum color intensity at 1.5—3.5 min after addition of 10% BrCN was measured at 405 mμ by a spectrophotometer (Hitachi EPU II) and was corrected for the blank absorbance. A plot of absorbances against concentration of nicotinic acid gave a straight line in the range

7) J.G.L. Harthon and J.T. Lindqvist, *Arzneimittel-Forsch.*, **14**, 1170 (1964).
8) K. Tsuji, T. Saito, and T. Komatsu, Abstract of Papers, the 84th Annual Meeting of Pharmaceutical Society of Japan, Tokyo, April 1964, p. 97 (6E27).
9) C.O. Badgett and C.F. Woodward, *J. Am. Chem. Soc.*, **69**, 2907 (1947).
10) A. Mueller and S.H. Fox, *J. Am. Pharm. Assoc., Sci. Ed.*, **40**, 513 (1951).

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of 0.1 mg/100 ml (8.12×10^{-6}M) to 1.5 mg/100 ml (1.22×10^{-4}M) with an absorbance of 1 mg/100 ml, 0.440. The percent liberation of nicotinic acid was calculated from a ratio of concentration of nicotinic acid at any time to the total calculated concentration of nicotinic acid liberated from IHN or the other nicotinic acid esters of polyhydric alcohols.

Titrimetric Procedure——Ten-milliliter aliquots of partially solvolyzed IHN solution were taken accurately using a syringe burret equipped with a micrometer. It was then titrated under nitrogen purge with 0.1N NaOH by the Radiometer titrator TTTlc with Titrigraph SBR2c (Radiometer, Copenhagen, Denmark) after previous addition with 9.5 ml of 0.1N NaOH under violent stirring. The amount of nicotinic acid formed in acidic solution was calculated from the difference between the alkaline titre of a sample solution in any time and the alkaline titre of an initial sample solution.

Thin-Layer Chromatographic Procedure——The degradation of IHN in 0.1N HCl at 37.0° was monitored by a thin-layer chromatography (TLC). The TLC plates were prepared with 0.4 mm layer of silica gel containing a fluorescent indicator (Wakogel B-5F, Wako Pure Chem. Industry, Ltd. Osaka). Concentrated NaOH solution was added to a small amount of the degraded solution so that pH of the solution was adjusted to 2.0—2.3, and 10 μl of this solution was spotted on the TLC plate. The TLC plate was then developed in a mixture of chloroform–isopropyl alcohol (7:3) up to about 15 cm. The spots separated on the plate were observed under 2537 Å ultraviolet light. IHN and all the reaction products were also detected with BrCN vapor after spraying 1% benzidine in ethanol.[11] IHN and partially degraded esters were detectable by spraying a mixture containing equal volumes of 10% $NH_2OH\cdot HCl$ and 20% NaOH, and subsequently by spraying a ferric nitrate reagent.[12]

The TLC technique was also used to quantitatively determine the amount of IHN in the solvolyzed IHN solution. The sample solution (5 ml) taken from 3.33×10^{-3}M IHN degraded in acidic solution at 37.0° was adjusted to pH 2.0—2.3 with NaOH solution in a 10-ml volumetric flask and made up to volume with distilled water. Five milliliters of this sample solution was added to 5 ml of chloroform in a glass-stoppered tube and shaken for 10 min and centrifuged for 5 min. The undegraded IHN was quantitatively extracted into the chloroform layer. Two-hundred microliters of the chloroform layer was streaked across a 0.4-mm thick silica gel plate. The silica gel (about 150 mg) at the *Rf* value (0.62—0.65) corresponding to IHN under observation with the ultraviolet light was scraped off for assay. The silica gel collected was extracted with 10 ml of chloroform–isopropyl alcohol (7:3) and the extract was used for the spectrophotometric analysis at 264 mμ. Approximately the same weight of silica gel scraped from a control plate was treated in a similar manner and used as the spectrophotometric blank. Eight milligrams of IHN was dissolved in 50 ml of chloroform, and appropriate dilution with chloroform was made to construct the standard curve in the range of 4×10^{-4}M to 2×10^{-3}M of IHN. Two-hundred microliter aliquots of the solution were then streaked across a 0.4-mm thick silica gel plate. A plot of absorbance against concentration of IHN which was extracted with chloroform–isopropyl alcohol (7:3) from silica gel collected after the developing gave a straight line with an absorbance of 3.33×10^{-3}M, 0.430. The recovery of IHN at the extraction was 99.7 ± 0.3%.

Stability in Enzymatic Solution——An enzymatic solution was prepared by mixing 900 mg of pancreatin powder with 30 ml of distilled water. After standing for 15 min with occasional shaking, the mixture was centrifuged, and the supernatant extract was used as the source of the enzyme. A mixture of 10 ml of the supernatant extract and 20 ml of distilled water was used as the pancreatin solution. An aqueous IHN suspension was prepared by adding 50 mg of IHN in 30 ml of distilled water or the pancreatin solution, and kept at pH 8.5 and 37.0° under a constant stirring in a jacketed titration vessel of the Radiometer titrator. Two-milliliter aliquots were taken out at different intervals with a pipette attached to a fritted-glass filter tube of No. 3 porosity and 10 mm diameter to remove particles of insoluble IHN. The sample solutions were transfered to flasks containing 4 ml of distilled water and 4 ml of 15% trichloroacetic acid. The flasks were shaken vigorously for 15 min, and centrifuged. The supernatant solutions were appropriately diluted with 0.1N HCl and analyzed spectrophotometrically at 261 mμ.

Dissolution Procedure——A similar method of Levy and Hayes[13] was used. The products tested were a brand of plain IHN tablet (200 mg) and of plain acetylsalicylic acid tablet (500 mg, J.P. VII). Two IHN tablets or one acetylsalicylic acid tablet were placed in 250 ml of 0.1N HCl which was contained in a 500 ml beaker and equilibrated to 37.0 ± 0.1°. A 3-bladed, 23-mm diameter stainless stirrer was rotated at a rate of 60 rpm. Seven-milliliter aliquots of the sample solution were taken out at 20, 50, 80 and 120 min with a pipette attached to the fritted-glass filter tube. Five milliliters of the sample solution was used for determination of liberated nicotinic acid by the ion-exchange chromatography and of IHN by the TLC, and 2 ml was used for determination of the total nicotinic acid after complete solvolysis by heating at about 90° with 4 ml of 0.5N NaOH. Seven-milliliter aliquots were withdrawn for the dissolution test of the acetylsalicylic acid tablet. The sample solutions were appropriately diluted with 0.1N HCl and analyzed spectrophoto-

11) S.O. Brattgard, R. Brattsand, and J.G.L. Harthon, *Arzneimittel-Forsch.*, **16**, 145 (1966).
12) M.E. Tate and C.T. Bishop, *Can. J. Chem.*, **40**, 1043 (1962).
13) G. Levy and B.A. Hayes, *New Engl. J. Med.*, **262**, 1053 (1960).

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metrically at 275 and 302 mμ. The total amounts of acetylsalicylic acid dissolved were calculated from sum of concentrations of acetylsalicylic acid and salicylic acid using simultaneous equations.[14]

The amounts of the compounds dissolved at each time of sampling were calculated appropriately by considering the decreased volume of the dissolution medium after repeated sampling and the amounts of the compounds removed at each sampling. The results of the dissolution study are expressed as $A_t/A \times 100\%$, where A_t is the amount at time t, and A is the amount at infinite time.

Solubility of IHN——Fifty-four milligrams of IHN (3.33×10^{-3}M) dissolved in 20 ml of 0.1N HCl was placed in titration vessel of the Radiometer titrator, and kept at specified pH with 0.1N NaOH. It was stirred at 37.0° for 1 hr, since it was considered that the solvolysis of IHN occurring during longer periods of time necessary to solubility equilibrium might lead to serious errors in the determination of IHN. Five milliliters of the sample solution was taken after removing the insoluble IHN powder, and adjusted to pH 2.0—2.3 by addition of 5 ml of HCl solution in an appropriate concentration. The resultant solution was added to 10 ml of chloroform in a glass-stoppered tube. The mixture was shaken for 10 min and centrifuged for 5 min. The chloroform layer was used for the determination of the solubility of IHN. The solubility of IHN was spectrophotometrically determined at 261 mμ using a standard curve in the range of 0.4 to 2.4 mg/100 ml-chloroform with a molar absorptivity, 18040. The solubilities of IHN so obtained are only approximate, since the possibility of supersaturation may present in this procedure.

Fifty milligrams of IHN was added in 20 ml of 0.1M NaCl in the titration vessel to determine the solubility of IHN at pH 8.0. The solution was kept at pH 8.0 and 37.0° under stirring until a saturate solution was obtained. Five-milliliter aliquots of the sample solution were withdrawn at intervals, and treated in a similar manner as in the acidic solution to determine the amount of IHN dissolved.

Results

Quantitative Determination of Nicotinic Acid in the Presence of Nicotinic Acid Esters by an Ion-Exchange Procedure

Quantitative determination of nicotinic acid liberated from *meso*-inositol hexanicotinate (IHN) in acidic solution was conducted by an ion-exchange chromatography. Of the anionic resins investigated, a weak basic resin pretreated by 1M acetate buffer solution at pH 4.9 was found to give a satisfactory separation of nicotinic acid from IHN partially solvolyzed. Nicotinic acid was quantitatively absorbed at pH 4.9 by the weak basic exchange resin (Amberlite CG-4B), and was determined in 0.3N hydrochloric acid-effluent by the reaction with cyanogen bromide and ammonia.

IHN and slightly soluble compounds in water, which appeared as the solvolysis proceeded, were required to be removed from the sample solution prior to the chromatographic separation, since these compounds precipitated at pH 4.9 and interfered with the chromatographic separation of nicotinic acid. Thus, an equal volume of chloroform to the sample solution adjusted to pH 2.0—2.3 was used to extract these compounds. Nicotinic acid remained in the aqueous layer in the pH range.

Preliminary tests conducted at the resin column (9×40 mm) packed into a chromatographic tube showed that the partially solvolyzed esters produced from 2.5 ml of 4.93×10^{-3}M (400 mg/100 ml) IHN in 0.1N hydrochloric acid were entirely eluted with distilled water into a 100 ml-volumetric flask, and nicotinic acid in 2.5 ml at a concentration of 2×10^{-2}M (246 mg/100 ml) was completely absorbed to the resin.

A mixture of 5 ml of 4×10^{-3}M nicotinic acid in distilled water and 1 ml of 1M acetate buffer solution at pH 4.9 was passed through the column. The nicotinic acid absorbed was not eluted with water alone until the tenth 10 ml-fraction and subsequently was eluted with 0.3N hydrochloric acid from the column. Ratios of the amounts of nicotinic acid to the total nicotinic acid eluted in successive five 10 ml-fractions were 0, 0.74, 99.08, 0.17 and 0%, and the total recovery of nicotinic acid was 99.99%. Thus, it was found that the nicotinic acid was completely eluted with 0.3N hydrochloric acid into a 50 ml-volumetric flask. Recovery tests of nicotinic acid was shown in Table I-A. The results of the determination of nicotinic

14) H.H. Willard, L.L. Merritt, and J.A. Dean, "Instrumental Methods of Analysis," 3rd ed., Princeton, New Jersey: Van Nostrand, 1958, p. 120.

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acid added to various partially degraded IHN solutions at 37.0° were shown in Table I–B. These tables indicated that satisfactory recoveries were obtained.

Table I. Ion-Exchange Chromatographic Determination of Nicotinic Acid

(I–A)

Added[a] mg/100ml	Found mg/100ml	Recovery %	
13.00	12.80	98.5	Av. 99.1
	12.93	99.3	
	12.93	99.3	
26.00	25.70	98.8	99.5
	25.82	99.3	
	26.10	100.3	
40.00	39.53	98.8	99.3
	39.80	99.5	
	39.80	99.5	

(I–B)

Time[b] hr	Formed[c] NA mg/100ml	Added[d] NA mg/100ml	Sum[e] Calcd. concn. mg/100ml		Found mg/100ml	
5	30.47	30.64	30.56	Av. 30.55	31.07	Av. 30.61
	30.38		30.51		30.42	
	30.52		30.58		30.33	
24	69.80	30.64	50.22	50.34	50.86	50.39
	70.17		50.40		50.39	
	70.17		50.40		49.93	
96	105.91	30.64	68.28	68.44	68.28	67.93
	106.15		68.40		67.89	
	106.62		68.63		67.66	

a) Nicotinic acid was dissolved in distilled water at each specified concentration, and 5 ml of each solution was used for the ion-exchange chromatography.
b) Time period of degradation for *meso*-inositol hexanicotinate (3.33×10^{-3}M) in 0.1 N HCl and at 37.0°.
c) Nicotinic acid concentration determined from 5 ml of the degraded *meso*-inositol hexanicotinate solution.
d) Nicotinic acid solution which was prepared for addition to the degraded sample solution shown at the left column.
e) Five milliliters of the degraded sample solution and 5 ml of the prepared nicotinic acid solution were mixed, and 5 ml of the mixture was used for the ion-exchange chromatography. Sum of nicotinic acid concentration was calculated from the concentrations of nicotinic acid formed and added.

The solvolysis of 4.93×10^{-3}M IHN in 0.1N hydrochloric acid at 37.0° was followed by measurement of nicotinic acid with the ion-exchange chromatography and the titrimetric method. The results of these analyses, as shown in Table II, indicated that a reasonable agreement was obtained. Nicotinic acid liberated from the other nicotinic acid esters of polyhydric alcohols was also found to be quantitatively determined by the ion-exchange chromatography.

Stability of Nicotinic Acid Esters of Polyhydric Alcohols in Acidic Media

The concentrations of nicotinic acid liberated from 3.33×10^{-3}M IHN in 1.00, 0.10 and 0.035N hydrochloric acid and at 37.0° against time was shown in Fig. 1. The concentration of nicotinic acid liberated was expressed in percentage of the total concentration of nicotinic acid liberated completely from the initial IHN concentration. The effect of the concentrations of hydrochloric acid on the formation rate of nicotinic acid at any time was not so re-

Table II. Comparison of Nicotinic Acid Concentration (mg/100ml) formed from *meso*-Inositol Hexanicotinate (400mg/100 ml) in 0.1 N HCl and at 37.0° by the Ion–Exchange Chromatography and the Titrimetry

Time hr	Ion–exchange chromatography		Titrimetry		Time hr	Ion–exchange chromatography		Titrimetry	
0	0.8	Av. 0.7	4.6	Av. 3.2	72	144.9	Av. 145.2	158.2	Av. 150.7
	0.5		1.6			144.5		152.2	
	—		3.3			146.1		141.8	
5	46.6	46.3	49.2	47.8	121	171.2	170.6	166.7	168.4
	45.6		45.0			170.3		166.4	
	46.8		49.3			170.3		172.0	
24	103.6	104.5	109.9	111.8	240	193.2	193.8	185.7	189.5
	104.4		112.4			192.7		188.7	
	105.4		113.0			195.5		194.2	

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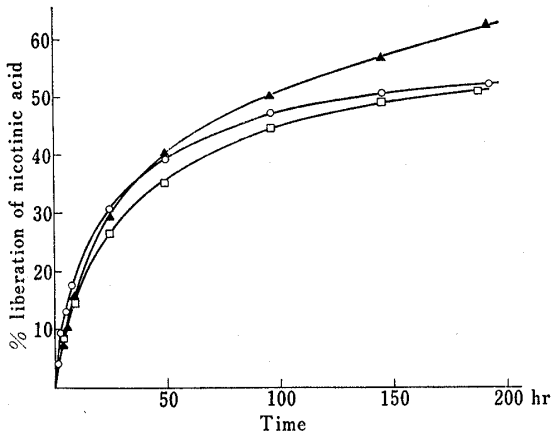

Fig. 1. Formation Curves of Nicotinic Acid liberated from *meso*-Inositol Hexanicotinate (3.33×10^{-3} M) at 37.0°

—□—: in 0.035 N HCl
—○—: 0.1 N HCl
—▲—: in 1.0 N HCl

Nicotinic acid concentration liberated was expressed in percentage of the total concentration of nicotinic acid completely liberated from *meso*-inositol hexanicotinate.

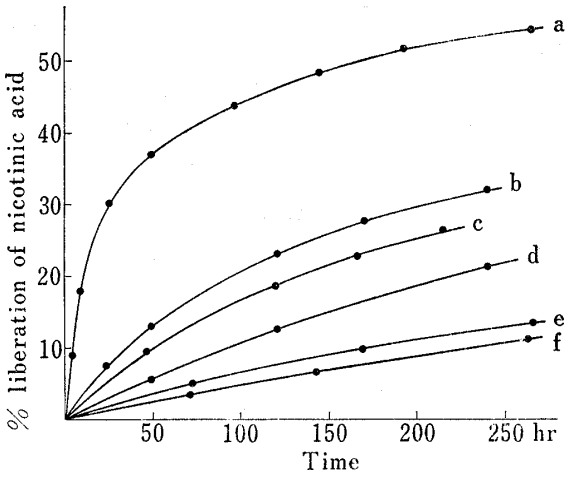

Fig. 2. Formation Curves of Nicotinic Acid liberated from Several Nicotinic Acid Esters of Polyhydric Alcohols in 0.1 N HCl and at 37.0°

The initial concentration of each ester was 2×10^{-2} M, when expressed as the total concentration of nicotinoyl groups.

a : *meso*-inositol hexanicotinate
b : mannitol hexanicotinate
c : erythritol tetranicotinate
d : glycerol trinicotinate
e : glycol dinicotinate
f : pentaerythritol tetranicotinate

markable as would be expected for ordinary carboxylic acid esters. On the contrary, the initial formation rate of nicotinic acid in 1.0N hydrochloric acid was slower than that in 0.1N hydrochloric acid.

The formation curve of nicotinic acid resulted from the initial IHN concentration at 1.11×10^{-3}M in 0.1N hydrochloric acid was identical to the curve at the same acid concentration in Fig. 1. Thus, it can be considered that the initial concentration of IHN has no influence on the shape of the formation curve of nicotinic acid expressed in percentage of the total concentration of the nicotinoyl groups.

The initial formation rate of nicotinic acid for IHN was compared with those for the several other nicotinic acid esters such as glycol dinicotinate, glycerol trinicotinate, erythritol tetranicotinate, pentaerythritol tetranicotinate and mannitol hexanicotinate in 0.1N hydrochloric acid. The studies were conducted at keeping the initial concentrations of these esters at 2×10^{-2}M, when expressed as the total concentration of the nicotinoyl groups. The concentrations of nicotinic acid liberated against time are shown in Fig. 2.

Table III. Initial Formation Rates calculated from the Formation Curves (Fig. 2) of Several Nicotinic Acid Esters of Polyhydric Alcohols in 0.1 N HCl and 37.0°

Compounds	Initial formation rates of nicotinic acid (%/hr)	Compounds	Initial formation rates of nicotinic acid (%/hr)
Glycol dinicotinate	0.063	Pentaerythritol tetranicotinate	0.046
Glycerol trinicotinate	0.103	Mannitol hexanicotinate	0.373
Erythritol tetranicotinate	0.341	*meso*-Inositol hexanicotinate	3.560

The initial concentrations of these esters were kept at 2×10^{-2} M, when expressed as the total concentration of nicotinoyl groups containing in these esters.

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Table III indicated that the initial formation rate of nicotinic acid for IHN was much faster than those for the other esters. However, the formation curve of nicotinic acid for IHN was, as shown in Fig. 2, in parallel with that of mannitol hexanicotinate after about 37% nicotinic acid liberation (about 50 hr). Thus, it was concluded that two or three nicotinoyl groups in IHN were much more reactive than those in the other esters.

Thin-Layer Chromatographic Analysis

The thin-layer chromatographic (TLC) plates spotted with the reaction mixture from 4.93×10^{-3}M IHN degraded in 0.1N hydrochloric acid were developed with chloroform–isopropyl alcohol (7:3). The results are given in Fig. 3.

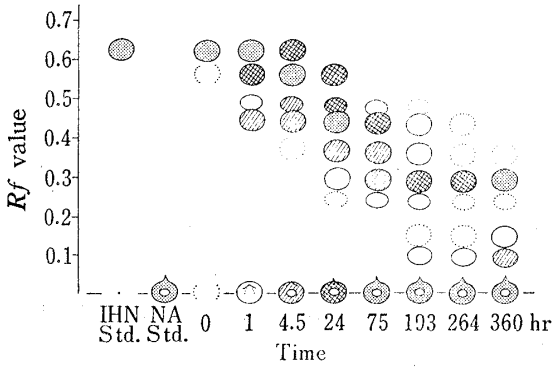

Fig. 3. Thin-Layer Chromatogram of *meso*-Inositol Hexanicotinate (4.93×10^{-3} M) degraded in 0.1 N HCl and at 37.0° as observed under Ultraviolet Light

Ten-microliter aliquots of the reaction mixture were spotted on the 0.4 mm-thick silica gel (Wakogel B-5F) plates and developed in chloroform–isopropyl alcohol (7:3). Intensities of the spots increase in the order of ○, ○, ○, ◉ and ◎ in each sample.

The intensity of a spot with an *Rf* value (0.62—0.65) corresponding to IHN in the zero hour sample decreased with time, while a spot corresponding to nicotinic acid which remained on the spotted point increased in intensity with time. The partially degraded esters were detected between IHN and nicotinic acid also by spraying ferric nitrate reagent after spraying of hydroxylamine and sodium hydroxide solution. The spots of three partially solvolyzed intermediates appeared in the early sample and intensified with time. The intensities reached maxima and then decreased with no corresponding spots at 360 hr. The spots detected were eight in this developing solvent except for IHN and nicotinic acid. It was assumed that IHN was solvolyzed to *meso*-inositol pentanicotinate which then successively decomposed to further degradation products, tetranicotinate, trinicotinate, dinicotinate and mononicotinate. Nicotinic acid is liberated from each step of the solvolytic reactions. Many stereoisomers are possible to exist for the partially solvolyzed nicotinic acid esters, suggesting that complex consecutive and parallel reactions are involved up to the completely solvolyzed products, nicotinic acid and *meso*-inositol. A similar mechanism on the degradation of IHN was suggested, and *meso*-inositol pentanicotinate was obtained from a degraded IHN solution by Nakanishi, *et al.*[15]

The quantitative determination of IHN with time was made by the TLC technique. For the quantitative determination, 200 μl of the sample solution extracted by chloroform was streaked across the silica gel plate, and the silica gel at the IHN zone was scraped from the plate after the developing, and the amount of IHN was determined. The acidic degradation of 3.33×10^{-3}M IHN monitored on the TLC plates was demonstrated by first-order plots. Typical first-order plots are shown in Fig. 4. The first-order rate constant of degradation of IHN in 0.1N hydrochloric acid and at 37.0° was calculated to be 1.86×10^{-1} hr^{-1} (half-life, 3.72 hr) from the slope of the plot of the logarithms of [IHN] against time in accordance with the following equation.

$$\log[\mathrm{IHN}] = -\frac{kt}{2.303} + \log[\mathrm{IHN}]_0$$

Where, $[\mathrm{IHN}]_0$ is the initial concentration of IHN at zero time.

15) M. Nakanishi, T. Kuriyama, and K. Arisono, Meeting of Kyushu, Pharmaceutical Society of Japan, Fukuoka, May 1966.

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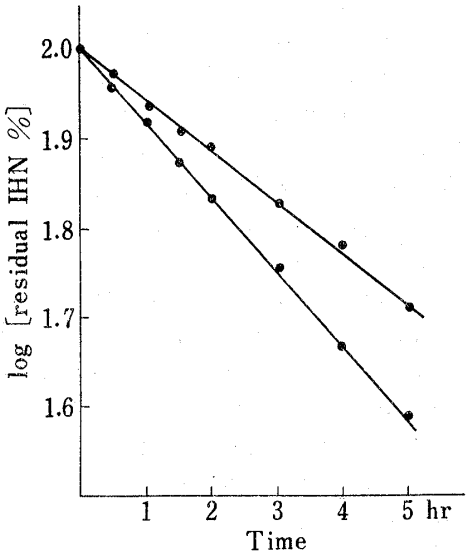

Fig. 4. Typical First-Order Plots for the Decrease of Concentration of *meso*-Inositol Hexanicotinate (3.33×10^{-3} M) degraded in 0.1 N HCl and 1.0 N HCl and at 37.0° by the Thin-Layer Chromatography

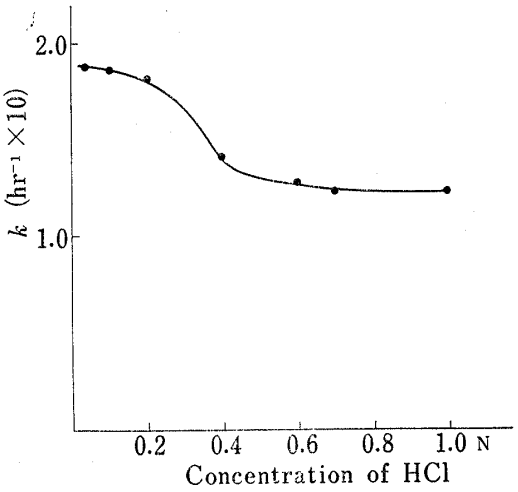

Fig. 5. Dependence of the Solvolytic Rate Constants of *meso*-Inositol Hexanicotinate (3.33×10^{-3} M) on Acidic Concentration at 37.0°

Dependency of the rate constant against hydrochloric acid concentration is shown in Fig. 5. The solvolytic rate constant of IHN in 1.0N hydrochloric acid was smaller than that in 0.1N hydrochloric acid, and the variation of the rate constants was minor in the range of 0.04 to 1.0N hydrochloric acid at 37.0°. This behavior of IHN on the degradation is different from that of ordinary carboxylic esters in acidic media. This is, however, reconciled with the results of the formation rates of nicotinic acid in the early stage shown in Fig. 1.

Dissolution Rate of *meso*-Inositol Hexanicotinate from Tablet

IHN is only slightly soluble in water, alkaline or neutral solution, but more soluble in acidic media. Therefore, it was assumed that absorption of IHN took place mainly in the stomach, and that a some extent of IHN was hydrolyzed prior to the absorption, but the amount of nicotinic acid liberated was not significant.[4] The dissolution rate of IHN from tablets and the gastric stability of IHN were examined by means of *in vitro* dissolution study in order to estimate the rate at which IHN was released from the tablet formulation. A similar method of Levy and Hayes[13] was used, and a commercial product containing 200 mg per tablet was used for the purpose of this study. Fig. 6 shows the dissolution behavior of the tablets.

An acetylsalicylic acid tablet (500 mg) was also subjected to the dissolution test in 0.1N hydrochloric acid for comparison, since it was presumed that acetylsalicylic acid was rapidly absorbed from the stomach.[16] The dissolution half-lives for two runs were less than 5 min, and these values were equal to about half the values shown in Levy's report.[13] It was shown that the IHN tablets used did not release its drug as readily as the acetylsalicylic acid tablet (a and b in Fig. 6), and about 5% nicotinic acid of the total bound nicotinic acid was liberated from the IHN up to 2 hr.

16) C.A.M. Hogben, L.S. Schanker, D.J. Tacco, and B.B. Brodie, *J. Pharmacol. Exptl. Therap.*, **120**, 540 (1957).

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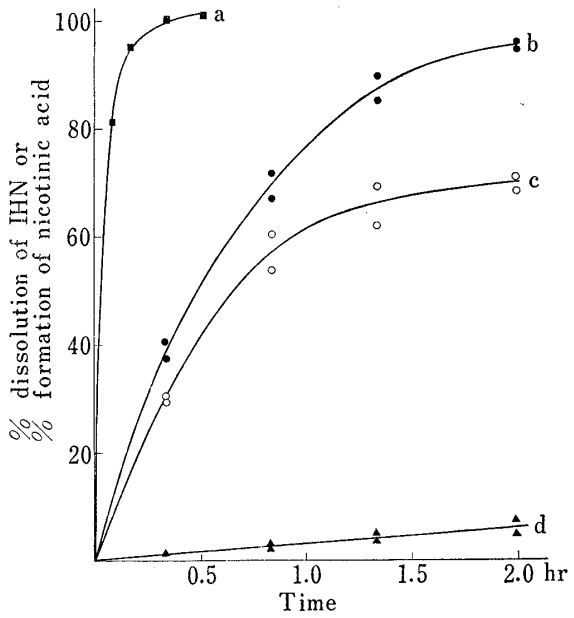

Fig. 6. *In Vitro* Dissolution of Commercial *meso*-Inositol Hexanicotinate Tablets (400 mg in 2 Tablets) and Acetylsalicylic Acid Tablet (500 mg in a Tablet) at 60 rpm in 0.1 N HCl at 37.0°

a : percent dissolution curve of acetylsalicylic acid (average for two runs)
b : percent dissolution curve of *meso*-inositol hexanicotinate calculated from the amount of nicotinic acid obtained after complete solvolyzation of sample solution by heating with NaOH solution
c : percent dissolution curve of *meso*-inositol hexanicotinate by the thin-layer chromatographic procedure
d : formation curve of nicotinic acid liberated during the dissolution test of *meso*-inositol hexanicotinate tablets Nicotinic acid liberated was expressed in percentage of the total nicotinic acid obtained from complete solvolyzation of *meso*-inositol hexanicotinate contained in the tablets.

The rate and extent of the drug dissolution from the tablets depend on the method of investigation and the brand of the tablet used. A number of methods[17] have been devised to test the release of drug from tablets. These methods approximately simulate *in vivo* condition. Levy[18] showed that the degree of agitation of the tablets by the dissolution method was greater than occurs in the stomach. Therefore, the amounts of IHN released and of nicotinic acid formed at any time in this dissolution test would presumably be overestimated for *in vivo* condition.

Discussion

Nicotinic acid liberated due to the degradation of *meso*-inositol hexanicotinate (IHN) in acidic solution can be accurately determined by the ion-exchange chromatography. The ion-exchange chromatographic procedure and the titrimetric assay conducted concurrently yielded comparable results for the partially solvolyzed IHN solution. However, the titrimetric assay requires a larger volume of the sample solution than the ion-exchange chromatographic procedure, and gives more scattered values than those by the latter method (Table II). A localized pH increase at unsufficient stirring may result in further solvolyses of the esters in the solution on adding sodium hydroxide solution, and the titre obtained may lead to considerable errors.

Vincke[5] reported that the solubility of IHN in water at pH 1.0—9.4 was considerably low so that the absorption from the gastrointestinal tract was not anticipated. Harthon and Lindqvist[7] and Chiti,[19] however, discussed that its solubility in 0.1N hydrochloric acid was sufficiently high (918 mg/100 ml) and that IHN was, therefore, presumed to be absorbed in the stomach after oral administration. Our experiment on the solubility of IHN at 37.0° indicated 180 mg/100 ml at pH 2.5, while it decreased with an increase of pH, 17.8, 6.7 and 1.24 mg/100 ml at pH 3.0, 4.0 and 8.0, respectively.

Analyses of enzymatic degradation of IHN were performed on pepsin in 0.1N hydrochloric acid (pH 1.2) and on pancreatin in aqueous IHN suspension at pH 8.5. The formation curve of nicotinic acid from IHN (3.33×10^{-3}M) in 0.1N hydrochloric acid containing 2.2% pepsin was not significantly changed as compared with that in 0.1N hydrochloric acid alone. No significant increase of ultraviolet absorbance at 261 mμ up to 2 hr was shown in aqueous IHN suspension and also in aqueous IHN suspension containing pancreatin kept at pH 8.5 and

17) J.F. Broadhead, A.G. Mitchell, and W.J. O'Reilly, *Aust. J. Pharm.*, **47**, S92 (1966).
18) G. Levy, *J. Pharm. Sci.*, **52**, 1039 (1963).
19) W. Chiti, *Farmaco* (Pavia), *Ed. Pract.*, **19**, 378 (1964).

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37.0° with the Radiometer pH stat device. Thus, the pancreatin is considered not to accelerate the degradation of the aqueous IHN suspension at pH 8.5. The solvolysis of IHN appears to be difficult probably due to its low solubility.

The result in Fig. 6 indicated that about 20% of IHN dissolved was solvolyzed, and the amount of nicotinic acid liberated was less than 5% of the total bound nicotinic acid up to 2 hr. The appearance of the partially solvolyzed esters may be favorable to the increase of solubility and absorption from the gastrointestinal tract, and the amount of nicotinic acid formed in the stomach would probably be not substantial as compared with the total amount of the bound nicotinic acid, although the solvolysis of IHN in 0.1N hydrochloric acid was considerably fast (Fig. 4).

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