[Chem. Pharm. Bull. 13(5) 551~557 (1965)] UDC 615.77: 615.724.8

74. Kiichiro Kakemi, Takaichi Arita, Hitoshi Sezaki, and Norio Takasugi: Absorption and Excretion of Drugs. XXII.*1 Absorption of Isoniazid and its Derivatives. (1).

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In the previous papers of this series, separatory determination of isoniazid and its metabolites in urine and inhibition of isoniazid acetylation by *p*-aminobenzaldehyde and its related compounds were reported.[1,2]

Drug absorption is a subject which is attracting increasing interest in the areas of pharmacy and pharmacology.[3] A better understanding of absorption can provide guide lines to the synthesis of new derivatives with better therapeutic response. It seems worthwhile, therefore, to investigate the relationship between the stability of a drug in the gastrointestinal tract and its absorption characteristics, when such drugs like isoniazid derivatives which contain a linkage susceptible to hydrolysis are taken orally.

The objectives of this project were to investigate the gastrointestinal absorption of isoniazid and its derivatives having anti-bacterial activity as a function of pH, compare chemical stability and absorption patterns, and to develop pharmaceutical procedures for enhancing physiological drug availability.

A great majority of the published work on the absorption and excretion of isoniazid and its derivatives has been directed towards the estimation of blood level and urinary excretion, and little attention has been paid to the possible hydrolysis and the absorption rate studies of isoniazid and its derivatives from the gastrointestinal tract at various pH values.[4~8]

In this report, the stability in the gastrointestinal tract of isoniazid derivatives, shown in Chart 1, was estimated from *in vitro* experiments in isotonic buffer solutions at 37° followed by the absorptin study from the gastrointestinal tract of rats under the condition where the degradation of the compounds was negligible. Effect of the gastrointestinal pH on the absorption of isoniazid and its derivatives is discussed. Support was obtained from the urinary excretion study of human urine after ingestion of glucose isonicotinoylhydrazone with or without glucose in favor of the result of perfusion experiment. It was also comfirmed that furyl methyl ketone isonicotinoylhydrazone, having equal or superior activity to isoniazid *in vitro* tests,[9] was readily absorbed in intact form from the gastrointestinal tract whereas rest of the hydrazones tested were poorly absorbed in intact forms.

A pharmaceutical technique, based on an understanding of this findings, which may render the drugs of this line more effective in clinical situation, is also discussed.

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*1 Part XXI: This Bulletin, **12**, 428 (1964).

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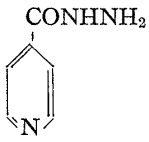
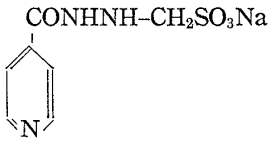
1) K. Kakemi, T. Arita, H. Sezaki, M. Nakano, T. Kiriyama: Yakugaku Zasshi, **82**, 195 (1962).
2) K. Kakemi, T. Arita, H. Sezaki, M. Nakano: *Ibid.*, **83**, 260 (1963).
3) J.G. Wagner: J. Pharm. Sci., **50**, 359 (1961).
4) D.F. Elmendorf. Jr., W.U. Cawthon, C. Muschenheim, W. McDermott: Am. Rev. Tuberc., **65**, 429 (1952).
5) B. Rubin, J.C. Burcke: J. Pharmacol. Exptl. Therap., **107**, 219 (1953).
6) H.B. Hughes: *Ibid.*, **109**, 444 (1953).
7) I. Yasuda: Niigata Igakukai Zasshi, **73**, 109 (1959).
8) Y. Shimomura: Kekkaku, **32**, 481 (1957).
9) K. Miyatake, S. Nagasaki, S. Ichimura, K. Hoji: Yakugaku Zasshi, **75**, 1066 (1955).

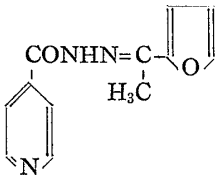
1) isoniazid (INH)



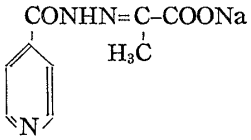
2) Na-(2-isonicotinoyl-hydrazino)-methanesulfonate (IHMS)



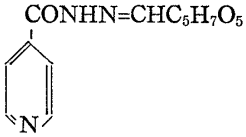
3) furyl methyl ketone isonicotinoyl-hydrazone (FKI)



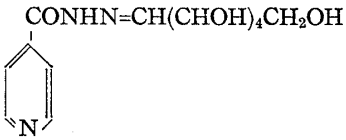
4) Na-pyruvate isonicotinoyl-hydrazone (P-INH)



5) gluculonolactone isonicotinoyl-hydrazone (GL-INH)



6) glucose isonicotinoyl-hydrazone (G-INH)



7) lactose isonicotinoyl-hydrazone (L-INH)

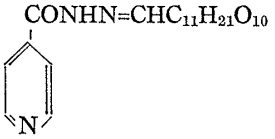


Chart 1. Isoniazid and its Derivatives

Experimental

Materials——Isoniazid (INH), sodium-(2-isonicotinoylhydrazino)-methanesulfonate (IHMS), and glucuronolactone isonicotinoylhydrazone (GL-INH) were obtained commercially. Lactose (L-INH) and glucose isonicotinoylhydrazone (G-INH) were prepared by the usual method.[10,11] Sodium pyruvate isonicotinoylhydrazone (Tanabe Seiyaku Co., Ltd.) (P-INH) and furyl methyl ketone isonicotinoylhydrazone (Daiichi Seiyaku Co., Ltd.) (FKI) were used as recieved. All other chemicals used were of reagent grade.

Equipments——A Shimadzu QV-50 spectrophotometer, Horiba M-3 pH meter (Hitachi-Horiba Instruments. Inc., Kyoto) and Taiyo thermounit C-100, (Taiyo Kagaku, Tokyo) were employed.

Analytical Procedure——Isoniazid concentrations were determined by the sodium 1,2-naphthoquinone-4-sulfonate method (method 1).[12]

Total isoniazid moiety concentrations were detemined by the cyanogen bromide method (method 2)[1] in terms of isonicotinic acid.

In kinetic studies, the anaiysis of IHMS in solution was conducted by running 1.0 ml. aliquot of the sample through a column of a weak cation exchange resin 5 ml. (Amberlite CG 50) in the hydrogen form to remove INH formed by the degradation. After the solution had passed below the upper level of the resin, the column was washed with distilled water to a volume of 50 ml. A 5 ml. aliquot of this solution

10) H. Zinner, W. Bock: Chem. Ber., **89**, 1124 (1957).
11) R. Yamamoto, H. Tanaka: Yakuzaigaku, **17**, 219 (1957).
12) H. Fujiwara: Yakugaku Zasshi, **78**, 1034 (1958).

was used for the assay by (method 2). The analysis of hydrazones in solution was conducted by (method 1) using 1.0 ml. aliquot of the sample and calculating the amount by measuring INH formed by the degradation of hydrazones.

In absorption studies, INH was determined by both method 1 and method 2 whereas IHMS was determined by method 2. The analysis of hydrazones in perfusate was conducted by method 2, modifying the step of oxidation by bromine solution as follows: for G-INH, 30 min., and for P-INH, 15 min., after treatment with one-tenth molar hydrochloric acid for 15 min. at room temperature.

Blood concentration of FKI was determined by diluting a 1.0 ml. whole blood with 0.2 ml. of 3.3 per cent sodium citrate solution in a syringe, and adding 0.4 ml. of 1 per cent solution of saponins to effect hemolysis. After 4 ml. of chloroform was added to the solution, the mixture was shaken vigorously for a few minutes and then centrifuged. The chloroform phase was separated, and optical density for intact FKI was determined at 310 mμ. The blood was taken immediately before drug administration and used for the assay blank by carrying out the same procedure. Compounds like acetylfuran, INH, and its metabolites, usually encountered in blood, did not interfere with the assay.

Kinetic Procedure——Appropriate amount of isoniazid derivatives was dissolved in water. An aliquot of the resultant solution was pipettrd into a 50 ml. volumetric flask which contained concentrated buffer solution and water, brought up to volume with water, mixed thoroughly, and immersed in a constant temrerature bath kept at 37°. One ml. of the drug solution was withdrawn at given time intervals, and the solution analyzed. The final concentrations of drug solution in these experiments were IHMS, 20 mg./100 ml.; GL-INH, 7 mg./100 ml.; L-INH, 10 mg./100 ml.; G-INH, 6 mg./100 ml.; P-INH, 6 mg./100 ml.; and FKI, 4 mg./100 ml. Same buffer systems, shown in Table I, were used for both stability and absorption studies. For the stability analysis of G-INH in the presence of excess glucose, the same procedure was carried at pH 2 and 6 by adding 0.5, 1, 2, 5 g. of glucose into a 50 ml. volumetric flask.

Table I. Isotonic Buffer Systems

pH	HCl (ml.)	Citric acid (g.)	$Na_2HPO_4 \cdot 12\,H_2O$ (g.)	KH_2PO_4 (g.)	NaCl (g.)	H_2O (g.)
1.2	9.5				4.0	
2.0	0.95				8.5	
3.2		22.1	26.5			to make
5.0		9.7	36.4			1000 ml.
6.0			4.3	7.5	5.8	
7.0			14.3	3.6	4.3	

Absorption from the Stomach and Small Intestine of Rats——The same procedure presented in the previous paper was followed.[13~16]

Absorption of G-INH with or without Excess Glucose——All experiments were conducted using adult humans in apparent normal health. A mixture of 460 mg. of G-INH and glucose (0, 5, 10 g.) was dissolved into 200 ml. of water. All drug solution were administered orally 30 min. after breakfast. Urine blanks were obtained immediately before drug administration. This value was usually negligibly small. Urine collection was carried out at given time intervals and INH excreted in urine was determined by the previous paper.[1]

Absorption of FKI from Enteric Coated Capsules——A mixture of FKI (or 40 mg. of FKI hydrochloride)*3 and an appropriate amount of potato starch was filled into a hard gelatin capsule (J. P. Ⅶ size No. 1). Capsules were coated one time with 15 per cent acetone solution of cellulose acetate phthalate. Each rabbit weighing 3~3.5 kg. was fasted overnight, and administered two of these capsules orally. No correction was made for body weight. One ml. of blood was taken from ear vein at given time intervals and used for assay. The control was taken as the ingestion of the same kind of non-coated capsules containing FKI or its hydrochloride.

Results and Discussion

The stability of isoniazid derivatives is summarized in Table Ⅱ. The stability of hydrazones in buffer solutions was found to decrease with lowering pH. If the pH of

*3 Particle passed through a 100 mesh screen.

13) K. Kakemi, T. Arita, S. Ohashi: Yakugaku Zasshi, 82, 348 (1962).
14) K. Kakemi, T. Arita, H. Sezaki, T. Nadai: *Ibid.*, 83, 871 (1963).
15) T. Koizumi, T. Arita, K. Kakemi: This Bulletin, **12**, 413 (1964).
16) *Idem*: *Ibid.*, **12**, 421 (1964).

Table II. Stability of Isoniazid Derivatives

	Initial concentration of drug solution (mg./100 ml.)	Per cent remaining of drug at one hour pH 1.2	pH 3.2	pH 5.0	pH 7.0	Half-life of drug at pH (time in min.)
IHMS	6	97	99	94	71	—
GL-INH	7	—	—	92	100	3.5
L-INH	10	—	—	96	100	24
G-INH	6	—	65	97	100	38
P-INH	6	—	—	41	100	>2
FKI	4	—	9	94	99	2

the stomach is assumed to be approximately 2, the stability of these drugs may be estimated from *in vitro* experiments at pH 2. The facile hydrolysis of these hydrazones to INH and its corresponding carbonyl compounds at the pH of the stomach should be taken into account for oral administration of these drugs.[17] While IHMS was stable at low pH and relatively unstable at neutral pH regions. Formation of isonicotinic acid and other degradation products was negligible.[18]

Both INH and IHMS were absorbed poorly from the stomach. INH was absorbed rapidly from the intestine. The absorption of IHMS from the small intestine was slower than that of INH. Result was obtained in favor of pH partition hypothesis that INH was absorbed more slowly at low pH, where the drug is ionized to a considerable extent. The good absorption of IHMS at pH 5 would partly be due to the conversion of IHMS to INH. The absorption study of IHMS at pH 7 was not carried out because of the degradation of IHMS to INH. These are shown in Fig. 1. The absorption of hydrazones from the small intestine of rats is given in Fig. 2. The absorption study of these hydrazones was carried out under the condition where the degradation of hydrazones to INH was negligible. Intestinal absorption patterns of these hydrazones are divided into two groups; slow and rapid absorptive ones. GL-INH, L-INH, G-INH,

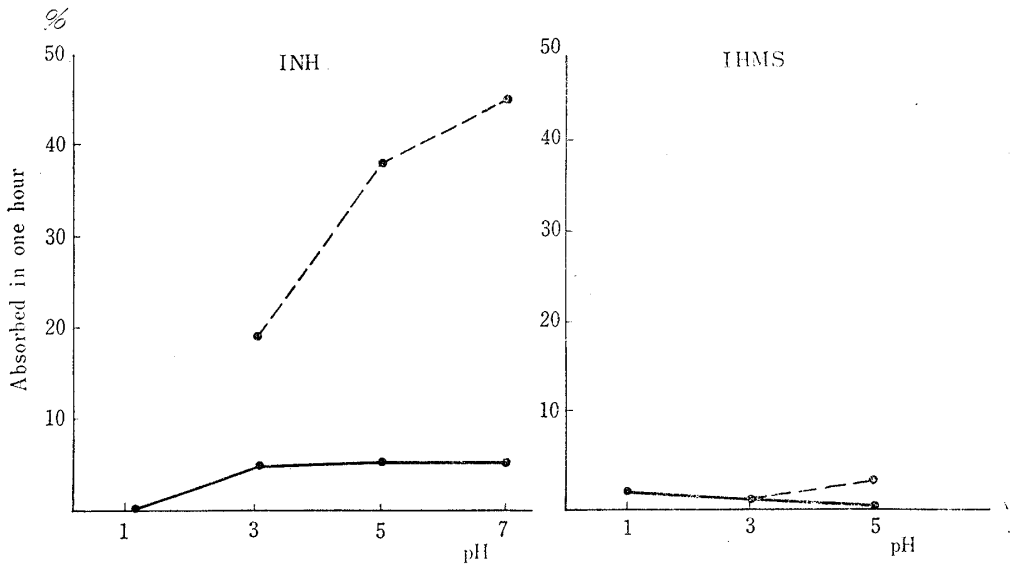


Fig. 1. Absorption of INH and IHMS

Stomach (————) Small Intestine (– – – – –)

17) E. R. Garrett: J. Pharm. Sci., **51**, 410 (1962).
18) Y. Mayuzumi: Seikagaku, **32**, 225 (1960).

and P-INH were hardly absorbed in intact form whereas FKI was absorbed in intact form to a considerable extent. The apparent chloroform/water partition ratio of these drugs in slow absorptive group were the order of 10^{-2}, while that of FKI was the order of 10. There is a rough relation between the degree of absorption of these drugs and their lipid solubility.[19] Some of the drugs in slow absorptive group once claimed to be less toxic and to have equal or superior antibacterial activity to INH. From the standpoint of drug availability after oral administration, however, there is a little doubt for the introduction of more polar carbonyl groups. The net absorption of L-INH and G-INH at pH 5 would be slightly smaller since possible degradation of these hydrazones to INH during experiments might be included. It has been discussed that there exhist correlation between pharmacological effects and the hydrolysis rates of isonicotinoylhydrazones.[20,21] When these hydrazones are ingested orally, pharmacological effects of the drugs might be attributed to INH liberated from hydrazones. This would be right, when hydrazones would not be absorbed in intact form and/or had inherently low pharmacological effects. These examples were GL-INH, L-INH, G-INH, and P-INH. While FKI was rapidly absorbed and had claimed to have a potent antibacterial activity,[9] this would not be acceptable.

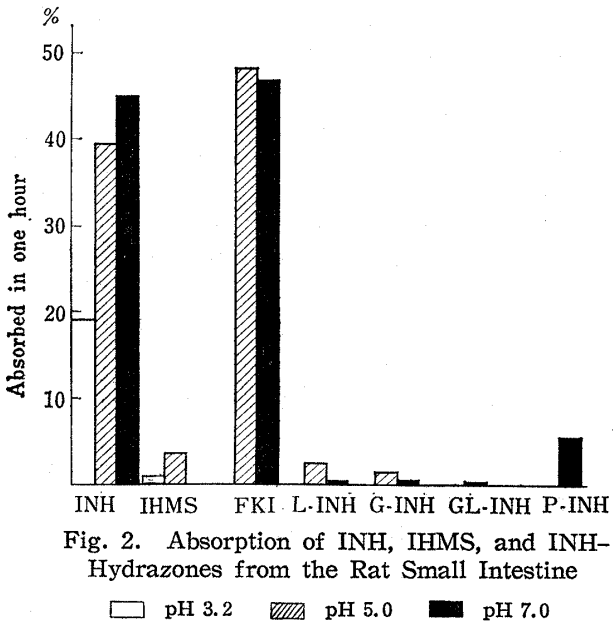


Fig. 2. Absorption of INH, IHMS, and INH-Hydrazones from the Rat Small Intestine

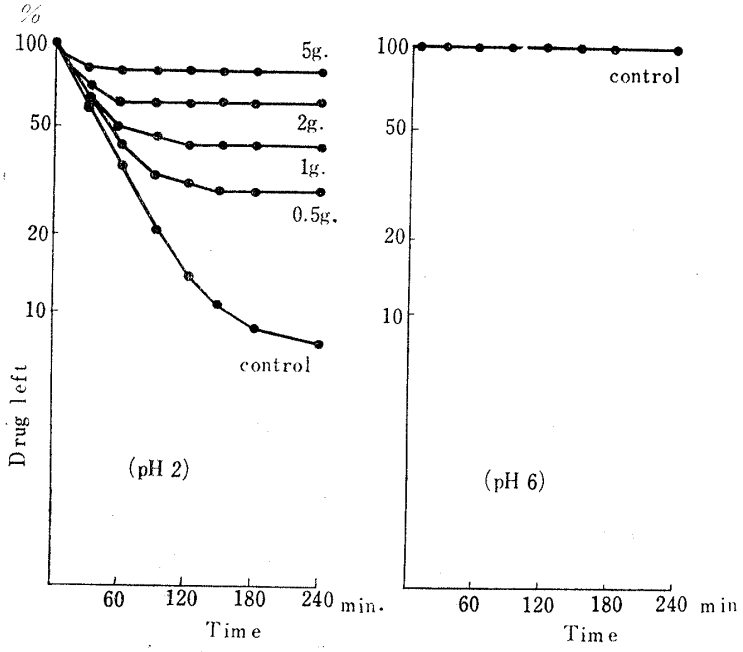


Fig. 3. Stability of Glucose Isonicotinoylhydrazone

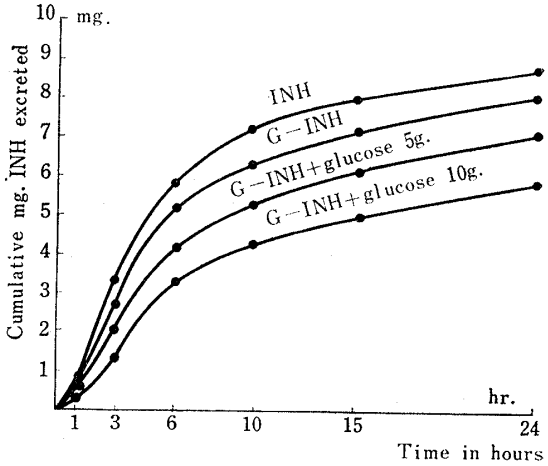


Fig. 4. Cumulative Excretion Curves for INH after Ingestion of Glucose Isonicotinoylhydrazone with or without Glucose

19) N. Nakagaki, K. Nara : Yakugaku Zasshi, **83**, 781 (1963).
20) *Idem* : *Ibid.*, **83**, 594 (1963).
21) H. Fujiwara : *Ibid.*, **78**, 1050 (1958).

Slow absorptive character of the hydrazones of the poorly absorptive group was further substantiated by the experiment with G-INH. The equilibrium of G-INH in solution is roughly expressed as follows. Glucose isonicotinoylhydrazone $\overset{H_3^+O}{\rightleftharpoons}$ isoniazid+glucose. As shown in Fig. 3, apparent hydrolysis rate of (G-INH) was suppressed by the addition of glucose. This was further demonstrated by the urinary experiment by humans. A typical graph of cumulated amount excreted in human urine *vs.* time is shown in Fig. 4. It was found that urinary excretion of INH decreased as the amount of glucose increased. Excretion of G-INH in human urine did not increased appreciably. Since the fraction of INH of the total isoniazid moiety excreted in urine was nearly constant in the subjects, decrease of INH in urine might safely be attributed to the inhibition of INH liberation in the gastrointestinal tract by the addition of excess glucose.

If FKI was taken orally, it would be rapidly changed into INH in the stomach and absorbed as INH. Since the absorption rate of FKI was equal or superior to that of INH and exceeds that of the other hydnazones in rats perfusion studies, and the antibacterial activity was compatible to that of INH, stabilization of FKI in the gastrointestinal tract would be highly effective from clinical standpoint. As a possible approach to the stabilization of FKI in the gastrointestinal tract, enteric coating technique was used. As shown in Fig. 5, high blood levels of FKI were obtained after

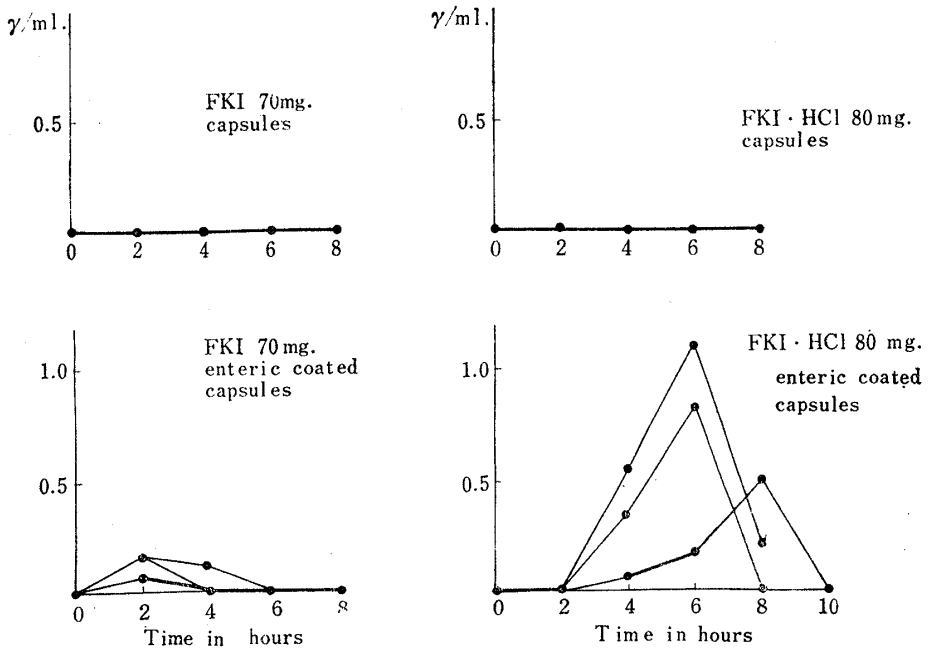


Fig. 5. Blood Concentrations of FKI following Oral Administration of FKI and FKI·HCl Preparations (Rabbit)

the oral administration of enteric coated capsules of FKI or its hydrochloride whereas no detectable amount of the drug was found in blood after the administration of non-coated capsules which contain the equal amount of the drugs.

The authors are indebted to Daiichi Seiyaku Co., Ltd. for their kind supply of the samples.

Summary

1) The stability of sodium (2-isonicotinoylhydrazino)methanesulfonate in isotonic

buffer solutions at 37° was found to be stable at low and relatively unstable at neutral pH regions.

2) The stability of isonicotinoyl hydrazones was found to decrease with lowering pH. The facile degradation of these compounds in the stomach should be taken into cosideration for oral administration.

3) Isoniazid was absorbed poorly from the stomach, and rapidly from the intestine. Sodium (2-isonicotinoylhydrazino)methanesulfonate was absorbed more slowly from the stomach and the intestine.

4) Intestinal absorption patterns of isonicotinoylhydrazones were divided into two groups; slow and rapid ones. Glucose, lactose, glucuronolactone, and sodium pyruvate isonicotinoylhydrazones were hardly absorbed in intact form whereas furyl methyl ketone isonicotinoylhydrazone was absorbed in intact form to a considerable extent. There was a rough relationship between the degree of absorption of these hydrazones and their lipid/water partition characteristics.

5) The fact that intact hydrazones of slow absorptive group are poorly absorbed was further demonstrated by the urinary excretion study using glucose isonicotinoylhydrazone by humans.

6) Suggestion was obtained to use isonicotinoylhydrazones belonging to rapid absorption group effectively by stabilization of the drug in the gut.

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75. Yoshihiro Nitta, Kiyoshi Okui, and Kiyohiko Ito: Pyrimidine Derivatives. I. Synthesis of a New Series of Sulfanilamides having Dialkylamino Groups in the Pyrimidine Nucleus.*1

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Up to the present time, a large number of sulfanilamidopyrimidines have been prepared in connection with research on new chemotherapeutic agents, and some of them have been used as medicines. Considering the history and development of the sulfanilamides, the authors have been greatly interested in changing the substituentes on the heterocyclic ring. For instance, sulfisomidine having two methyl groups on the pyrimidine ring was found to have stronger and broader antibacterial activities than sulfadiazine having no substituent, and had been used as an excellent sulfa drug in the early stage of the development. However, in 1956, sulfadimethoxine, in which the two methyl groups of sulfisomidine were replaced by two methoxy groups, was presented.[1] This replacement produced more beneficial change in the biological properties. Sulfadimethoxine is well absorbed, maintains prolonged blood concentration, displays a low degree of acetylation, has a low urinary excretion rate, and is less toxic. Consequently, the methoxy group was noted by many investigators as an effective substituent, resulting in the appearance of some valuable compounds such as N^1-(5-methoxy-

*1 Presented at the 83rd annual meeting of the Pharmaceutical Society of Japan (1963).

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1) W. Klötzer: Monatsh. Chem., 87, 131 (1956).