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The Influence of Bacterial Gut Hydrolysis on the Fate of Orally Administered Isonicotinuric Acid in Man[1]

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Following oral administration to human subjects, isonicotinuric acid was hydrolyzed within the gastrointestinal tract to isonicotinic acid. This metabolism did not occur following intravenous administration. Evidence is presented indicating that most of the ingested isonicotinuric acid escaped absorption from the small intestine and passed into the large intestine, where gastrointestinal bacteria hydrolyzed it to isonicotinic acid. The latter compound was subsequently absorbed into the systemic circulation, where some was reconjugated with glycine, forming the originally administered compound, isonicotinuric acid.

KEY WORDS: isonicotinuric acid; gastrointestinal transit; bacterial metabolism; biopharmaceutics; glycine conjugation.

INTRODUCTION

The review article in 1968 by Scheline (1) on drug metabolism by intestinal microorganisms was a prelude to a resurgence of interest in this area by microbiologists and biochemists. Consequently, over the past 6 years it has become increasingly apparent that gastrointestinal metabolism of compounds by bacteria may appreciably affect biological properties (2). Bacterial metabolism may occur after oral administration of a compound or as a consequence of diffusion or secretion of the compound or its metabolites into the gastrointestinal tract (2).

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This paper deals with the hydrolysis of the glycine conjugate isonicotinuric acid (INU). Cleavage of the amide bond of glycine conjugates by gastrointestinal bacteria has been reported by several investigators. Much of the work in this area has dealt with the deconjugation of the bile acid glycocholic acid. This conjugate is excreted via the bile into the small intestine where some escapes reabsorption from the ileum and passes into the colon. Therein the glycine moiety is removed by bacteria (3). This hydrolysis has been shown to occur in isolated cultures of bacteria belonging to the genera *Clostridium* (4–8), *Streptococcus* (4–8), *Aerobacter* (9), *Bacteroides* (5–8,10), *Veillonella* (5,7,8), *Bifidobacterium* (5,7,8), *Staphylococcus* (5,7,8), *Lactobacillus* (6), *Eubacterium* (6), *Catenabacterium* (6), *Ramibacterium* (6), *Butyribacterium* (6), *Alkaligenes* (6), and *Proteus* (6); additionally, bacterial enzyme preparations have been shown to catalyze glycocholate cleavage (7,11–16). Bile acid metabolism by bacteria has been reviewed by Lewis and Gorbach (17) in a concise tabular form.

Hydrolysis of other glycine conjugates has also been reported. Norman and Grubb (4) indicated that enterococci are known to be capable of hydrolyzing hippurate to benzoate. *p*-Aminohippuric acid is hydrolyzed to *p*-aminobenzoic acid when incubated with rat cecum extract (18) or with cultures of *Streptococcus faecalis* or *Aerobacter aerogenes* (19). Amide hydrolysis occurred when *p*-aminohippurate and *p*-acetylaminohippurate were administered orally to human subjects (20); hydrolysis did not occur following intravenous administration.

The present report deals with the hydrolysis of isonicotinuric acid in man by gastrointestinal bacteria. During the course of investigations dealing

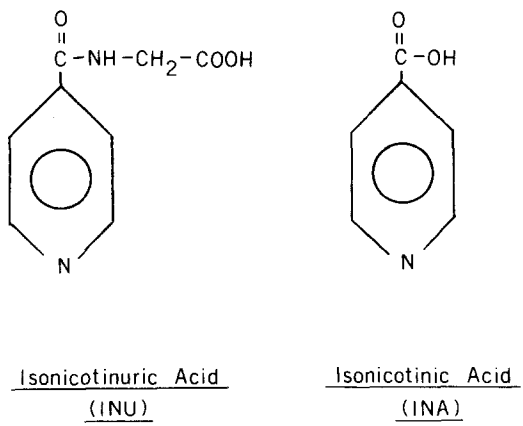

Fig. 1. Chemical structures of isonicotinuric and isonicotinic acids.

with the pharmacokinetics of isoniazid and some of its metabolites in man (21), isonicotinuric acid (INU) was administered orally and intravenously (i.v.) to human subjects. Following i.v. administration, INU was excreted quantitatively in the urine intact. Following oral administration, however, both INU and its hydrolysis product, isonicotinic acid (INA), were excreted in the urine. The structures of these two compounds are shown in Fig. 1. These experiments indicated that hydrolysis of the amide bond occurred within the gastrointestinal tract. It will be reported herein that following oral administration of INU to human subjects only a small amount of intact INU was absorbed from the upper gastrointestinal tract. The remaining INU then passed to the lower gastrointestinal tract, where bacteria hydrolyzed it to INA. This INA was absorbed from the gastrointestinal tract into the systemic circulation, whereupon it underwent distribution, metabolism, and excretion. Part of the absorbed INA was reconjugated with glycine and excreted into the urine as INU.

MATERIALS AND METHODS

Chemicals

Isonicotinuric acid was prepared by a modification of the method used by Rohrlich (22) for the preparation of nicotinuric acid. One normal NaOH was used in place of 0.1 N NaOH, and concentrated HCl was subsequently used to achieve neutralization. The product was recrystallized four times from 50% ethanol, mp 224–225°C dec. (corr.). Analysis calculated for $C_8H_8N_2O_3$: C, 53.33; H, 4.48; N, 15.55. Found: C, 53.10; H, 4.24; N, 15.68.

Isonicotinic acid (Eastman) was recrystallized thrice from water, mp 304–305°C (corr., sublimation).

Assay Procedure

INA and INU were converted to polymethine dyes and determined colorimetrically using the two-component analysis described by Boxenbaum and Riegelman (23). The sensitivity of the method permitted assay of urine samples and incubates, but not blood.

Potentiometric Titration

Potentiometric *p*H titration of INU was conducted following the recommended procedures of Benet and Goyan (24). The INU was titrated against both acid and base, and the data were analyzed using the computer program of Litchinsky *et al.* (25).

Subjects

Three adult male volunteers in apparent good health served as subjects. Subject A was a Caucasian male, age 30 years, weight 69.0 kg, and height 165 cm. Subject B was a Caucasian male, age 50 years, weight 80.0 kg, and height 181 cm. Subject C was a native of northern India residing in the United States, age 22 years, weight 54.5 kg, and height 175 cm. Subjects A and B lived on diets rich in animal fat and protein, whereas subject C lived on a diet devoid of animal protein but containing milk products.

Human Experiments

None of the subjects received any drugs for 1 month prior to the experiments, and experiments in any individual were conducted at intervals of at least 2 weeks. Unless otherwise indicated, all studies were conducted after an overnight fast, and INU was administered dissolved in 240 ml water. Water and food were withheld following INU administration for 1 and 3 hr, respectively, and then permitted *ad libitum*. Urine was collected at approximate half-hour intervals for 10–13 hr and then at convenient intervals until INA and INU could no longer be detected ($\geq$ 24 hr). During the initial 10–13 hr urine collection period, urine *p*H was determined immediately after voiding.

Each of the three subjects ingested 128–130 mg INU (studies 1, 2, and 3 for subjects A, B, and C, respectively). Whereas subjects B and C participated in this one experiment only, subject A participated in several additional studies. In study 4, the influence of a larger INU dose was investigated by subject A ingesting 476 mg INU dissolved in 500 ml water.

To investigate the influence of urinary *p*H control, subject A participated in two studies in which 325-mg sodium bicarbonate tablets U.S.P. were administered with water. In study 5, sodium bicarbonate tablets were administered with 120–240 ml water at 2.85 hr (13 tabs), 1.80 hr (13 tabs), and 0.67 hr (9 tabs) prior to ingestion of 129 mg INU. At the time of INU administration, urine *p*H was 8.2 and was maintained at 8.0 $\pm$ 0.2 by hourly bicarbonate ingestion (26). Bicarbonate supplementation was discontinued 9 hr after INU administration. Because bicarbonate administration prior to INU ingestion apparently influenced absorption, an additional experiment with controlled urinary *p*H was conducted with subject A. In study 6, sodium bicarbonate administration was begun 2 hr after ingestion of 129 mg INU. Thirteen bicarbonate tablets were ingested at 2, 2.5, 3, and 3.5 hr and then as required at half-hour intervals to maintain urinary *p*H at 8.0 $\pm$ 0.2 (26). Consequently, urine *p*H was controlled from 4 to 15 hr after INU ingestion.

The influence of an anticholinergic agent on absorption was investigated in study 7. Two 15-mg propantheline bromide tablets U.S.P. (Pro-Banthine,

Searle Laboratories, Chicago, Ill.) were administered with 30 ml water to subject A at 9 and 0.5 hr prior to ingestion of 130 mg INU. Following INU administration, one propantheline bromide tablet was taken every 3 hr for three doses.

Attempts were made to reduce bacterial gut populations by oral administration of two antibiotics, kanamycin and lincomycin. In study 8, subject A received kanamycin sulfate capsules U.S.P. (Kantrex-Bristol Laboratories, Syracuse, N.Y.—each capsule equivalent to 0.5 g kanamycin base) on a regimen of two every hour for four doses, then two every 6 hr around the clock for nine doses. Two and one-fourth hours after the last kanamycin capsules, 128 mg INU was ingested. In study 9, subject A received lincomycin hydrochloride capsules U.S.P. (Lincocin, Upjohn Company, Kalamazoo, Mich.—each capsule equivalent to 0.5 g lincomycin base) on a regimen of one capsule every 6 hr around the clock for 11 doses. This subject acquired diarrhea after the sixth dose, and feces returned to normal 28 hr after the last lincomycin dose. A dose of 130 mg INU was administered 5 hr before the last lincomycin dose.

In study 10, INU was administered to subject A by rectal retention enema shortly after defecation. The enema contained 1.00% INU and 0.576% NaCl in distilled water, and 4.90 ml (49 mg INU) was instilled rectally. The enema was retained, and further defecation occurred only subsequent to the disappearance of INA and INU from urine samples.

Incubation Studies

The *p*H stability of INU was studied under conditions similar to the *p*H extremes found within the gastrointestinal tract. INU (108 μg/ml) was incubated aerobically at 37°C for 5 hr in 0.100 N HCl and for 48 hr in 0.067 M tris buffer (*p*H 8.75).

Stability of INU to pancreatic enzymes was investigated using aqueous extracts of pancreatin (porcine, activity equivalent to 4 times N.F., Sigma Chemical Company, St. Louis, Mo.) as described by Glazko *et al.* (27). Solutions (*p*H 8.75) containing pancreatic enzymes, tris buffer, and INU (108 μg/ml) were incubated aerobically at 37°C, and aliquots were periodically withdrawn for assay up to 48 hr. Each 1-ml aliquot was diluted to 10 ml with 95% ethanol to precipitate proteins, and the clear filtrate was used for assay.

The stability of INU to fecal bacteria was investigated using an aqueous suspension prepared from the feces of subject A. Incubates were prepared according to Scheline (28) and contained INU (833 μg/ml), feces, phosphate buffer, glucose, yeast extract, and peptone. Aerobic incubations were conducted in 50-ml tubes stoppered with a plug of sterile cotton. Anaerobic

incubations were conducted in 12.5-ml culture tubes with air atop the tubes flushed with nitrogen. In some experiments, the incubates contained a mixture of chloramphenicol, oxytetracycline hydrochloride, and neomycin sulfate; each antibiotic was present at a concentration equivalent to 4.17 μg/ml free base. All incubations were conducted at 37°C.

RESULTS AND DISCUSSION

Disposition of Isonicotinic and Isonicotinuric Acids in Man

Before discussing the fate of INU following oral administration, it would be helpful to review the pharmacokinetics of INA and INU following intravenous administration. Boxenbaum and Riegelman (21) administered INA and INU to subjects A and B. The doses of approximately 200 mg INA and 120 mg INU were infused intravenously at a constant rate over a period of 1 hr; urine was collected and its *p*H was maintained at a value of 8.0 $\pm$ 0.2 by oral administration of sodium bicarbonate (26). Following i.v. administration of INU, the intact compound was excreted quantitatively in the urine. When INA was administered, both intact compound and its glycine conjugate (INU) were excreted in the urine; recovery of these two compounds in urine accounted for 100% of the i.v. doses. On the basis of these experiments, it was concluded that INU is not hydrolyzed within the body (excluding the gastrointestinal tract) and that INA is metabolized to INU. Appropriate pharmacokinetic analysis led to the model illustrated in Fig. 2; average first-order rate constants for the two subjects are shown. Elimination of INA from the peripheral compartment, determined by the

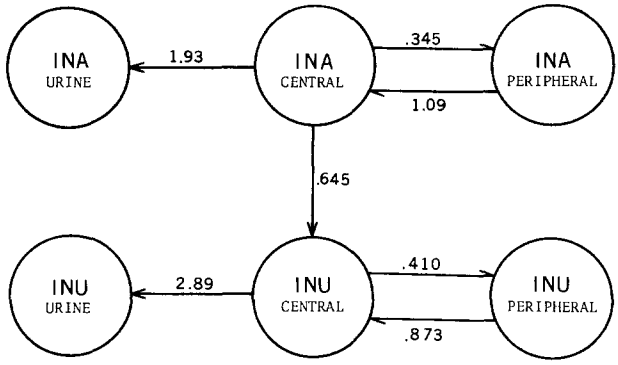

Fig. 2. Pharmacokinetic model for the disposition of isonicotinic and isonicotinuric acids in man. Arrows represent first-order processes, and numbers are average first-order rate constants (hr^{-1}).

method of Rowland *et al.* (29), was found to be less than 1%; this was considered quantitatively negligible, and most probably differed from zero only as a result of experimental error.

Rate of Excretion Curves Following Oral Administration of Isonicotinuric Acid

Figures 3 and 4 show rate of excretion curves for INU and INA following oral administration of 128–130 mg INU; also shown are the curves following a 476-mg oral dose to subject A. Ellard and Gammon (30) administered 250 mg INA orally to a human subject and determined plasma renal clearance values of 453 and 493 ml/min for INA and INU, respectively. Consequently, rate of excretion data in any particular study would be expected to directly reflect plasma levels.

These and subsequent plots may be more fully understood by comparing rates of excretion to rates of absorption. The Loo–Riegelman equation (31) for urinary excretion data was used to calculate $f \cdot (A)_{tn}$, where f is the fraction of compound reaching the circulation which is excreted unchanged in the urine and $(A)_{tn}$ is the cumulative amount of compound absorbed at time tn. These data were used to calculate $f \cdot (\Delta A/\Delta t)$, where $(\Delta A/\Delta t)$ is an approximation of the rate of absorption at the midpoint of the absorption time interval. Rate of excretion plots for INU and INA are compared to $f \cdot (\Delta A/\Delta t)$ plots in Fig. 5. Aside from a slight shift of the time axes, the curves are virtually superimposable. This is a consequence of the relatively large values for the disposition parameters of both INU and INA; half-lives of terminal exponential phases of INU and INA following i.v. injection are approximately 0.95 and 0.76 hr, respectively.

Cursory examination of the plots in Figs. 3 and 4 indicates that a limited amount of INU is rapidly absorbed, with peak rates of excretion occurring within $\frac{1}{4}$–$1\frac{1}{2}$ hr following administration. Unabsorbed INU, presumably as it continues down the gastrointestinal tract, is converted to INA; this compound is subsequently absorbed and once in the body is excreted intact and metabolized to INU. The reconversion of INA to INU and subsequent excretion of INU are responsible for the second peak in the INU rate of excretion curves. The terminal exponential half-life $(t_{1/2})_\beta$ for INA reported by Boxenbaum and Riegelman (21) is approximately 0.76 hr; therefore, the downward slope of these INA rate of excretion curves during the 5–30 hr period is much less steep than would be expected had INA absorption ceased. Thus it appears INA absorption continued for as long as 30 hr after the initial INU dose.

Eve (32) reviewed much of the literature dealing with gastrointestinal transit, and recommends 1 and 4 hr, respectively, for the mean transit times

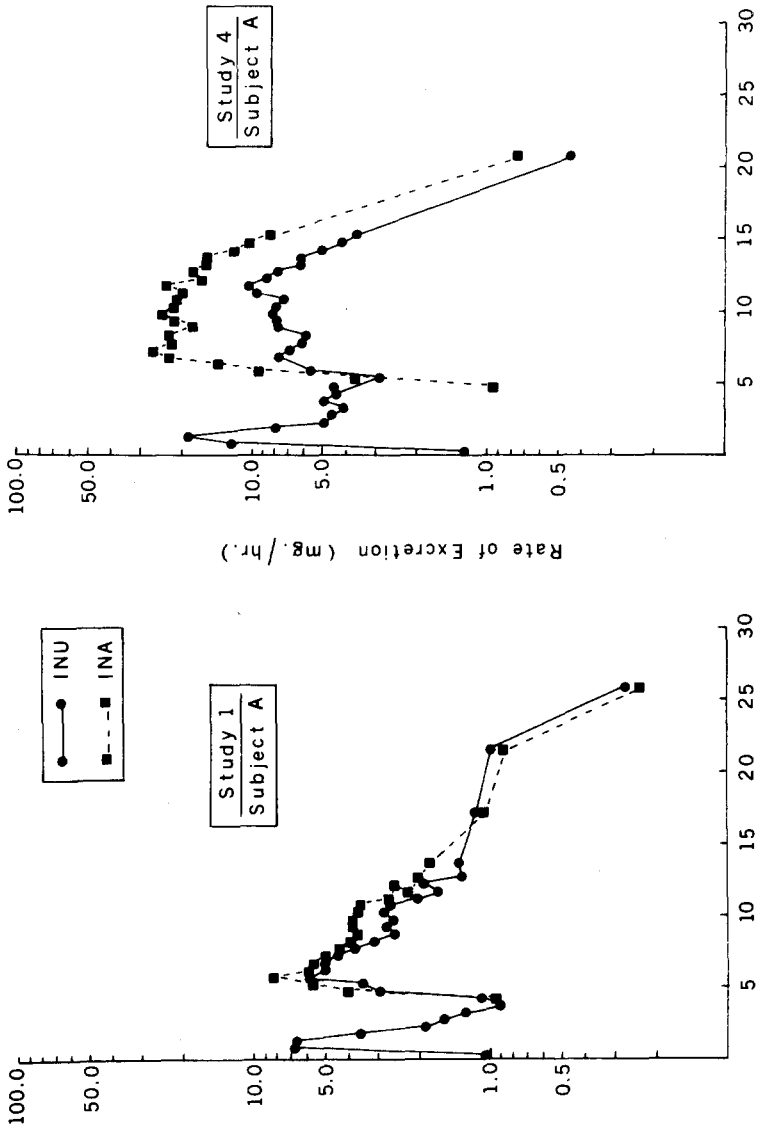

Fig. 3. Rate of excretion curves following oral administration of isonicotinuric acid to subject A. Isonicotinic acid rates expressed in terms of mole equivalents of isonicotinuric acid.

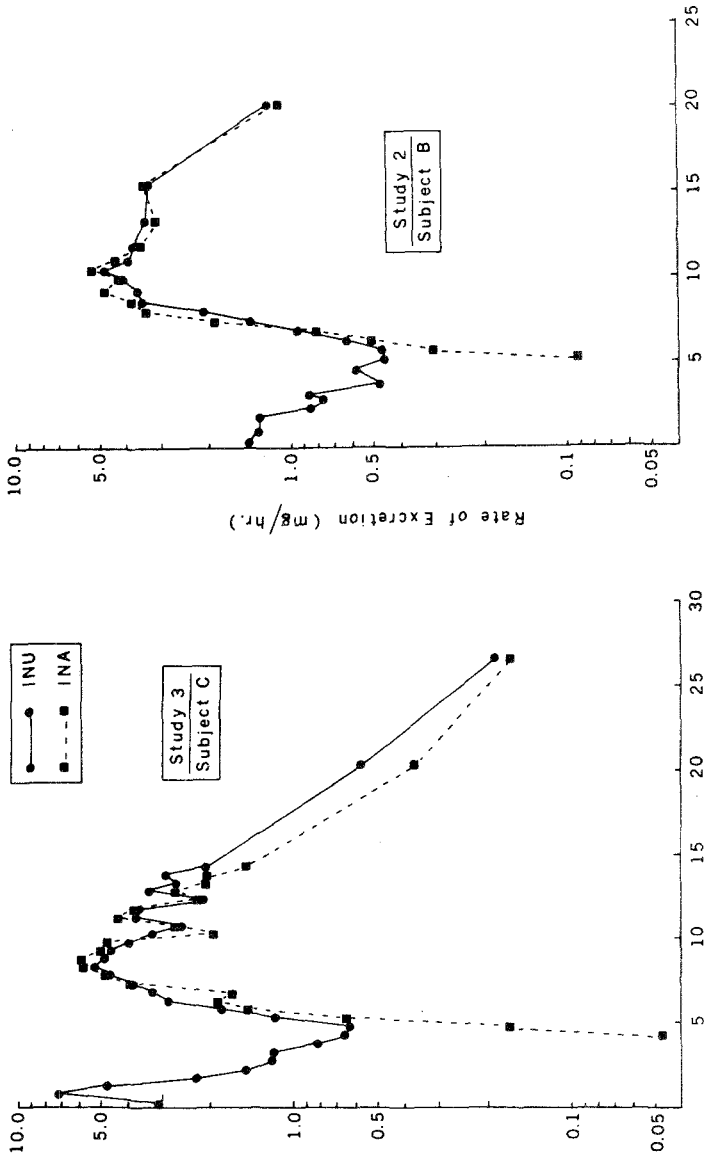

Fig. 4. Rate of excretion curves following oral administration of isonicotinuric acid to subjects B and C. Isonicotinic acid rates expressed in terms of mole equivalents of isonicotinuric acid.

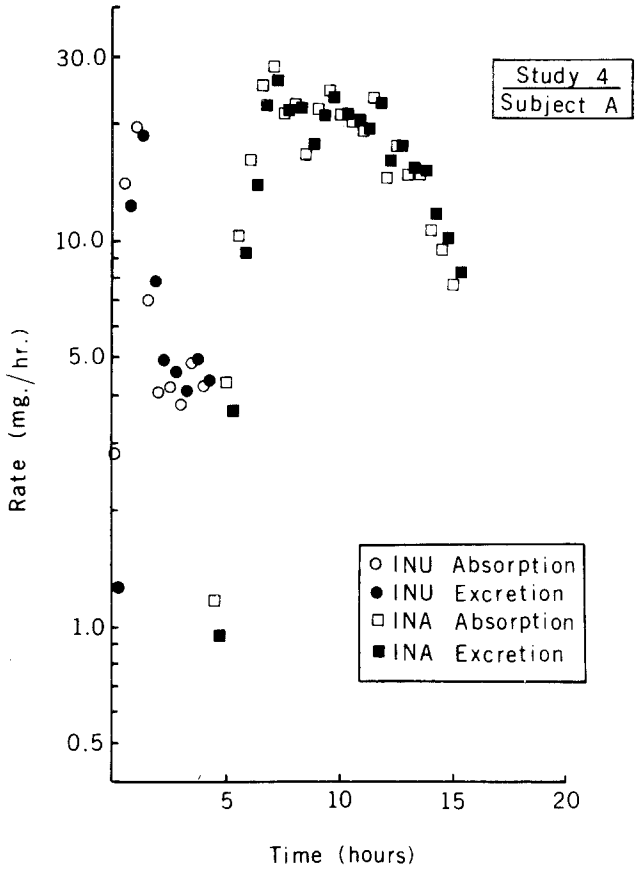

Fig. 5. Relationship between rates of absorption and excretion of isonicotinuric and isonicotinic acids following oral administration of isonicotinuric acid to subject A; absorption rates are actually $f \cdot (\Delta A/\Delta t)$ (see text for explanation). Isonicotinic acid rates expressed in terms of mole equivalents of isonicotinuric acid.

of food through the stomach and small intestine of humans. Eve reported that following an ordinary barium meal the average time taken by the head of the meal to reach the cecum was $1\frac{1}{2}$–2 hr, and that times between 1 and 4 hr should be considered normal. The time required for all the barium to pass through the terminal ileum was observed to be 6 hr, although other investigators have reported 8 hr or longer. In the present experiments, therefore, it seemed reasonable to assume that INU which escaped absorption from the small intestine passed on to the cecum and more distal parts of the large intestine where it underwent bacterial hydrolysis; the INA so formed

was subsequently absorbed. The curves for the vegetarian subject C in Fig. 4 are very similar to those for subjects A and B, who ate meat. Thus there is no evidence that diet affects the fate of orally administered INU.

Incubation Experiments

Incubation experiments at 37°C with INU in 0.1 N HCl and *p*H 8.75 buffer indicated no hydrolysis or decomposition for at least 5 and 48 hr, respectively. The most acidic portion of the gastrointestinal tract is the stomach (*p*H $\approx$ 3.0), whereas the most alkaline segment is the ileum (*p*H $\approx$ 7.5) (33). It may therefore be concluded that the *p*H of the luminal contents of the gastrointestinal tract was not responsible for INU hydrolysis.

Incubation of INU at 37°C with a pancreatic extract (*p*H 8.75) indicated complete stability for at least 48 hr. The conditions for preparation and testing activity of the extract were those used by Glazko *et al.* (27) for enzymatic hydrolysis of chloramphenicol esters. While this particular extract may have had enzymatic activity toward chloramphenicol esters, it is quite possible that experimental procedures either masked or destroyed INU hydrolysis activity. Indeed, the *p*H of 8.75 of the final preparation was greater than that normally found within the gastrointestinal tract. A more critical experiment would have been to incubate INU with fresh, membrane-sterilized, pancreatic or other intestinal juices. The present experiment, therefore, while lending no support to pancreatic enzymatic activity as a mediator of INU hydrolysis, does not exclude the possibility of such activity *in vivo*. However, as shall be discussed subsequently, activity by digestive enzymes seems highly unlikely because pretreatment of subject A with the antibiotic lincomycin completely eliminated INU hydrolysis in the gut.

Evidence supporting mediation of INU hydrolysis through intestinal bacteria is found in the fecal incubation studies summarized in Table I. It has been determined that at least 20% of the mass of feces consists of live bacteria (33). In excess of 99% of the total cultivable fecal flora of normal adults are obligate, nonsporing anaerobes, consisting predominantly of bifidobacteria (anaerobic lactobacilli) and bacteroides (33–36). Enterobacteria (e.g., *Escherichia coli*), usually thought of as typical fecal organisms, contribute less than 0.1% of the total bacterial population, and enterococci and clostridia are only minor components of the flora (33). In view of the nature of fecal flora, the results of INU fecal incubation studies are not surprising. Not more than 5% of the INU was hydrolyzed when incubated 12 hr aerobically with feces, whereas, anaerobically, hydrolysis proceeded to greater than 90% completion after 12 hr. At 52 hr, however, aerobic incubation of INU with feces resulted in complete hydrolysis. Antibiotics reduced the extent of hydrolysis, but did not completely block it; higher

Table I. Hydrolysis of INU by Fecal Incubations

Aerobic incubations	Percent recovery			
	At 12 hr		At 52 hr	
Sample contents	INU	INA	INU	INA
Medium + feces (blank)	0	0	0	0
Medium + INU	99.0	0	90.6	5.26
Medium + INU + feces	95.0	2.95	0	96.7
Medium + INU + feces + antibiotics	97.7	0.868	68.7	35.7
Medium + INA + feces	0	103	0	102

Anaerobic incubations	Percent recovery at 12 hr	
Sample contents	INU	INA
Medium + INU	98.5	0
Medium + INU + feces	2.80	91.8
Medium + INU + feces + antibiotics	92.6	4.41
Medium + INA + feces	0	96.3

levels than those employed (4.17 μg/ml) might have further reduced or completely prevented bacterial hydrolysis. Bacteria did not appear to further degrade INA.

It is important to recognize that the bacterial culture methods were faulty from a bacteriological point of view. Anaerobic incubations, for example, would better have been conducted in a "Gas-Pak" or one of the other types of anaerobic jars or chambers (37). Additionally, the use of selective media to promote growth of desired organisms and retard the growth of unwanted organisms could possibly have yielded more meaningful data.

Influence of Antibiotic Pretreatment on Isonicotinuric Acid Gut Hydrolysis

Since fecal incubation studies suggested that gastrointestinal tract bacteria were responsible for INU gastrointestinal hydrolysis, oral kanamycin and lincomycin were administered to subject A with the hope of sufficiently reducing or eliminating those intestinal bacterial populations responsible. Administration of either antibiotic did not affect the urine assays of INA and INU.

The results of pretreatment with kanamycin and lincomycin on the fate of INU are illustrated in Fig. 6. Kanamycin showed virtually no effect, whereas lincomycin completely prevented INU hydrolysis. These experiments provide a clue as to the nature of the microflora responsible for the

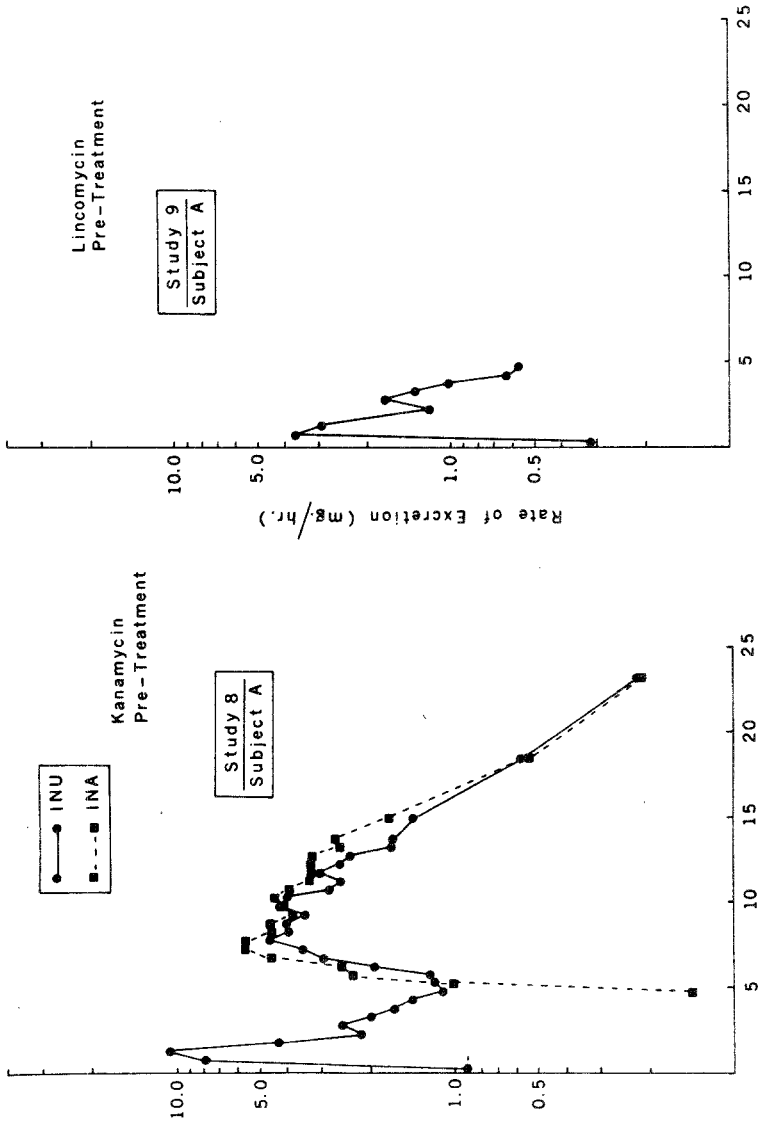

Fig. 6. Influence of antibiotic pretreatment on rate of excretion curves following oral administration of isonicotinuric acid to subject A. Isonicotinic acid rates expressed in terms of mole equivalents of isonicotinuric acid.

hydrolysis. As reviewed by Scheline (2), anaerobic bacteria can be largely eliminated with little or no change in the aerobes with lincomycin (38,39), whereas the opposite result can be largely achieved with kanamycin (40). This suggests that anaerobes were responsible for INU hydrolysis. A more meaningful conclusion regarding the relative roles of aerobic and anaerobic bacteria could have been made if good quantitative aerobic and anaerobic cultures of the subject's feces had been made before and after antibiotic therapy.

It may be concluded from the lincomycin study in Fig. 6 that intact INU was incompletely absorbed, and that absorption occurred only from the more proximal segments of the gastrointestinal tract. Thus, of the 124-mg oral dose, only 7.38 mg was absorbed; absorption ceased after 5 hr, and INU which proceeded down the gastrointestinal tract was not absorbed. This is in contrast to INA, which can be absorbed from distal portions of the gastrointestinal tract. The reason for incomplete INU absorption was not ascertained, but could have resulted from a capacity-limited absorption mechanism or as a consequence of only a narrow segment of the intestine being capable of absorbing INU. Another possibility to explain the incomplete absorption would be an unfavorable *in vivo* partitioning of INU between gastrointestinal membrane and luminal contents.

Another interesting result from lincomycin pretreatment was reduced INU absorption. In study 1 without lincomycin, approximately 12.2 mg INU was absorbed intact, whereas with lincomycin only 7.38 mg was absorbed. This could have been the result of day-to-day variation or possibly due to lincomycin pretreatment. Spanknebel *et al.* (41) found in ileostomy patients that oral lincomycin increased ileal effluent *p*H and bicarbonate output. The possibility exists that lincomycin-induced electrolyte changes may have diminished INU absorption. Analogously, Jacobson *et al.* (42) found that oral neomycin produced a broad spectrum of malabsorptive difficulties.

Rectal Administration of Isonicotinuric Acid

Experiments suggested that INU was hydrolyzed by bacteria within the large intestine and that liberated INA was subsequently absorbed. Support of this hypothesis comes from study 10, in which INU was administered by rectal retention enema to subject A. Figure 7 shows INU and INA rates of excretion, and it may be concluded that INU is rapidly hydrolyzed to INA, which is subsequently absorbed. A small amount of INU is absorbed intact, as indicated by the INU rate of excretion far exceeding that for INA in the first few data points. It may be remembered that INU absorption ceased after 5 hr in the lincomycin study, indicating that absorption did not occur from the rectum. In the lincomycin study, however, the INU

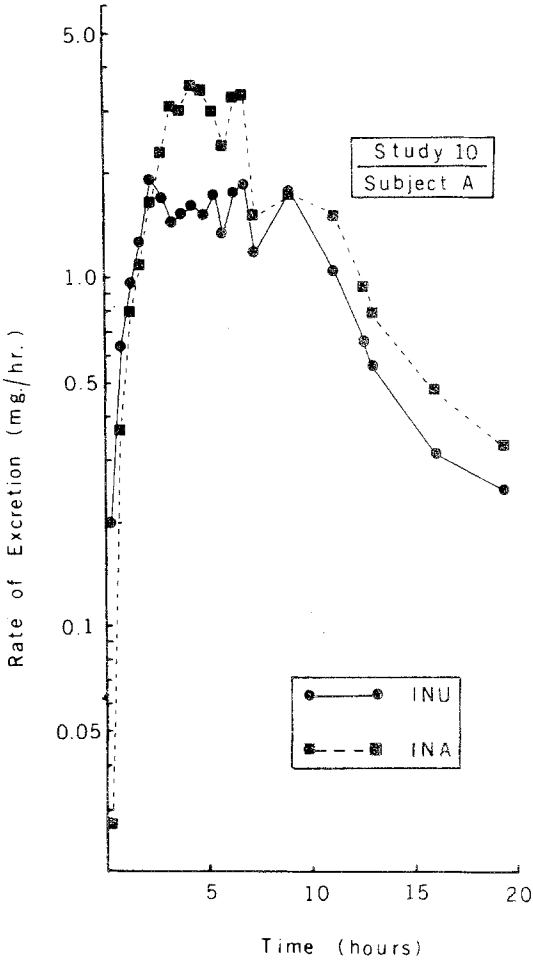

Fig 7. Rate of excretion curves following rectal administration of isonicotinuric acid to subject A. Isonicotinic acid rates expressed in terms of mole equivalents of isonicotinuric acid.

probably passed into the large intestine and rectum and dispersed within the luminal content; this may have been responsible for its lack of absorption. The urinary assay of INU is quite sensitive, and could have detected INU urinary levels resulting from absorption rates of less than 0.6 mg/hr.

Influence of Sodium Bicarbonate Administration on the Absorption of Isonicotinuric and Isonicotinic Acids

The influence of controlling urinary *p*H by administration of sodium bicarbonate is shown in Fig. 8. Pretreatment with bicarbonate prior to

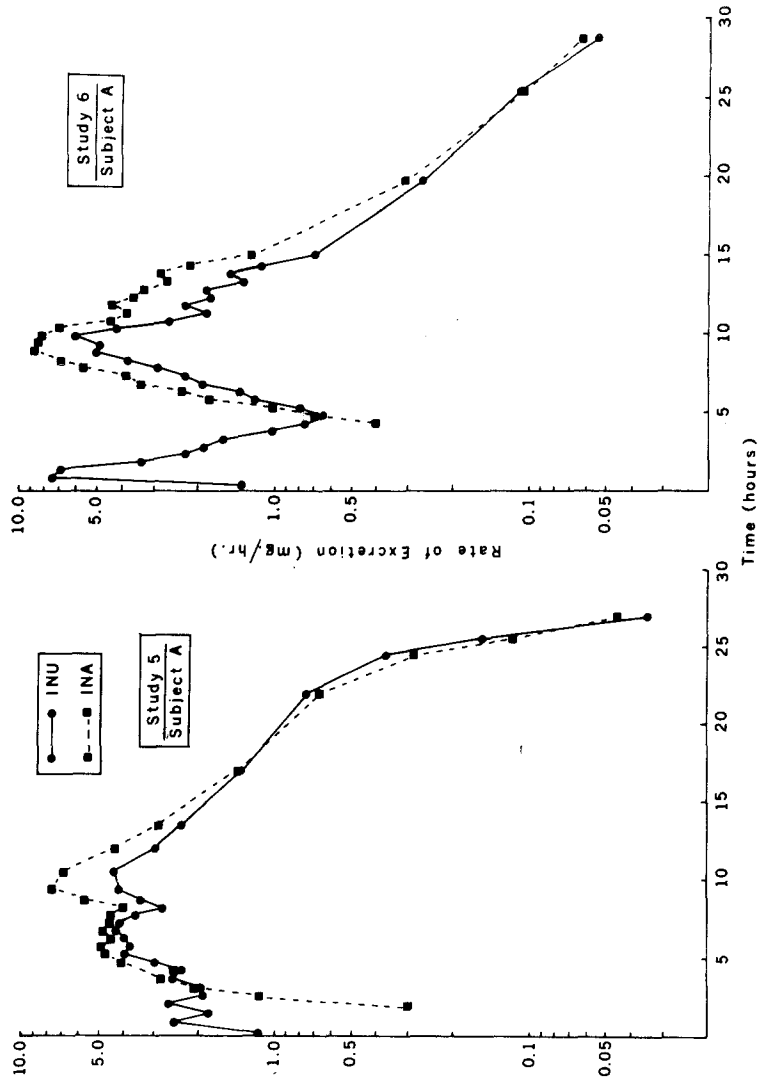

Fig. 8. Influence of sodium bicarbonate administration on rate of excretion curves following oral administration of isonicotinuric acid to subject A. Isonicotinic acid rates expressed in terms of mole equivalents of isonicotinuric acid.

administration of INU resulted in erratic INU absorption, which was not observed when bicarbonate administration was begun 2 hr subsequent to INU ingestion. Boxenbaum (43) reported that INA excretion rates were affected by changes in urine *p*H and that these fluctuations could be eliminated by controlling urine *p*H with bicarbonate. Thus fluctuations in rates of excretion in Fig. 8 reflect fluctuations in absorption rates.

Enhancement of Isonicotinuric Acid Absorption

Two studies were conducted in an attempt to increase the amount of INU absorbed intact. In study 4 (Fig. 3), a larger dose (476 mg) was administered. In this study, 34.3 mg was absorbed intact compared to 12.2 mg when 129 mg was administered to the same subject. In study 7, the administration of the anticholinergic agent propantheline also affected INU absorption. In this study, subject A had a dry mouth and difficulty in urinating, indicating satisfactory propantheline absorption. Figure 9 shows rate of excretion curves indicating enhancement and prolongation of INU absorption. INU absorption appeared to continue for a few hours longer than in study 1, and the initial appearance of INA in urine was delayed from 3.75 hr in study 1 to 6.25 hr in the propantheline study. Additionally, whereas 12.2 mg INU was absorbed intact in study 1, 37.2 mg INU was absorbed intact in the propantheline study. The increased bioavailability of intact INU most probably resulted as a consequence of the pharmacological activity of propantheline; this anticholinergic agent reduces gastric motor activity and secretion and inhibits gastrointestinal motility (44–47). Another interesting aspect of Fig. 9 is the influence of propantheline on INA absorption. The INA rate of excretion curve has two distinct peaks. A similar pattern is present in other studies, but the effect here is much more pronounced. Again, this most probably resulted from the influence of propantheline on gastrointestinal motility.

Levy *et al.* (48) reported an enhancement of riboflavin absorption similar to that observed for INU; propantheline increased riboflavin absorption an average of 2.2-fold. Increased absorption due to propantheline was attributed to prolonged contact of the vitamin with specialized absorption sites in the small intestine. A similar enhancement of phenolsulfonphthalein absorption by propantheline was reported by Ashley and Levy (49). It may be therefore, as was suggested for riboflavin (48), that INU may be absorbed only from particular sites in the small intestine. It is of particular interest that the INU excretion rate curves (and consequently the absorption rate curves) are very similar in shape to concentration–time curves of nonabsorbable markers as they move through segments of the gastrointestinal tract (50–53). It may be that INU absorption rate is highly dependent on

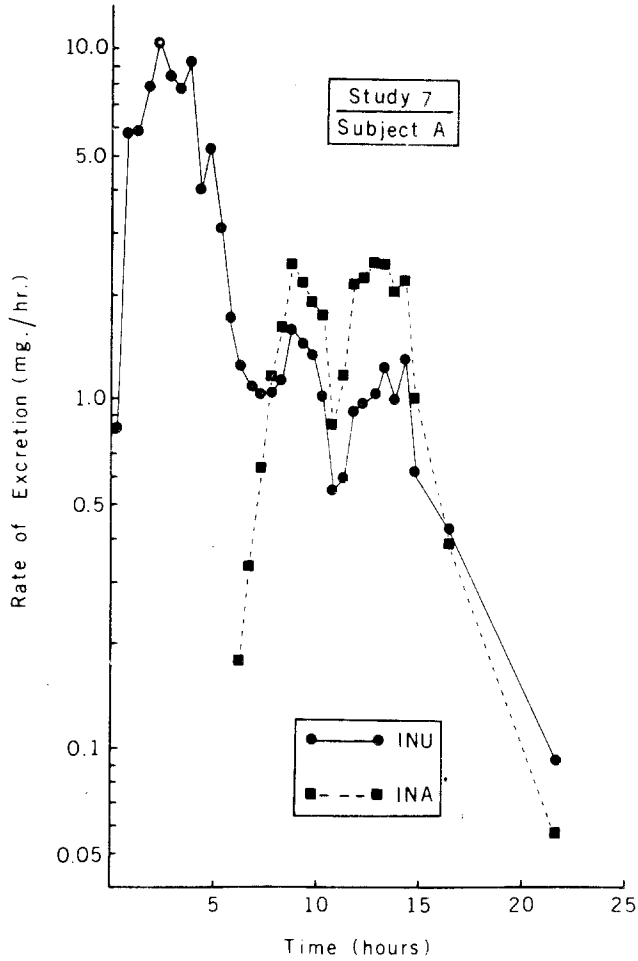

Fig. 9. Influence of propantheline administration on rate of excretion curves following oral administration of isonicotinuric acid to subject A. Isonicotinic acid rates expressed in terms of mole equivalents of isonicotinuric acid.

gastrointestinal flow rate through a particular segment of the gastrointestinal tract.

Dissociation of Isonicotinuric Acid in the Small Intestine

An aqueous solution containing 0.005 M INU and 0.045 M KCl had a *p*H value of 3.20, indicating dissociation of the carboxylic acid moiety. This solution was titrated against 0.005 N NaOH, and the stoichiometry of

the titration curve indicated one titratable group. Appropriate analysis of the titration curve (25) indicated a thermodynamic pK_a of 3.86 for the carboxylic acid function. Titration with 0.005 N HCl showed no titratable group, indicating the pyridinium moiety is a very weak base. Thus at *p*H values associated with the small intestine (*p*H 6–7.5) (33), INU exists predominantly as the carboxylate ion. The thermodynamic pK_as of INA are approximately 1.75 and 4.90 (54), and it too exists in the small intestine predominantly as the carboxylate ion. Ellard and Gammon (30) administered INA orally to human subjects, and peak plasma levels occurred at approximately $\frac{1}{2}$ hr; approximately 95% of the dose was recovered in urine as INA and INU. Thus ionization of INA in the small intestine did not limit its absorption, and presumably ionization of INU in these experiments was not primarily responsible for its poor absorption characteristics.

Relationships Between Rate of Excretion, Gastrointestinal Transit, and Microbial Populations

Eve (32) published an extensive review of gastrointestinal transit including recommendations of mean passage times of food residues through the stomach, small intestine, upper large intestine, and lower large intestine. Subsequently, Bernard and Hayes (55) presented a four-compartment, catenary model for gastrointestinal transit, shown in Fig. 10. The four compartments of the gastrointestinal tract were taken to be the stomach, small intestine, upper large intestine, and lower large intestine. Transfer was assumed to be first order and unidirectional, and the reciprocals of Eve's mean passage times through the various segments (compartments) were taken to be the first-order rate constants. No doubt the model has numerous shortcomings and is extraordinarily simplistic. One obvious shortcoming is that elimination from the lower large intestine (defecation) is taken to occur continuously. Additionally, the model parameters were selected to mimic gastrointestinal transit of unabsorbed food residues in an average man consuming an average diet. Movement of an unabsorbed compound taken after an overnight fast would probably occur more rapidly. For example, the half-life of gastric emptying is taken as 41.6 min; volumes

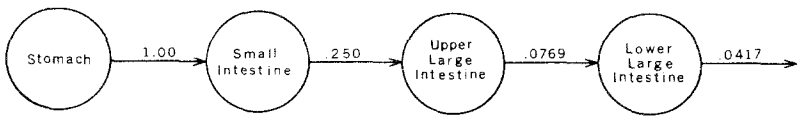

Fig. 10. Catenary model illustrating gastrointestinal transit of food residues. Arrows represent first-order processes, and numbers represent first-order rate constants (hr^{-1}).

of 250 and 500 ml water empty from the stomach with a half-life of approximately 7 min (56), and a 750-ml pectin meal empties with a half-life of approximately 21.8 min (57). Thus it seems likely that the ingested INU solutions proceeded down the tract at a faster rate than that predicted from the model. Nonetheless, the model does provide at least some means of estimating residues in the gastrointestinal tract as a function of time. Figure 11 utilizes the model and shows the fraction of unabsorbed residue in the

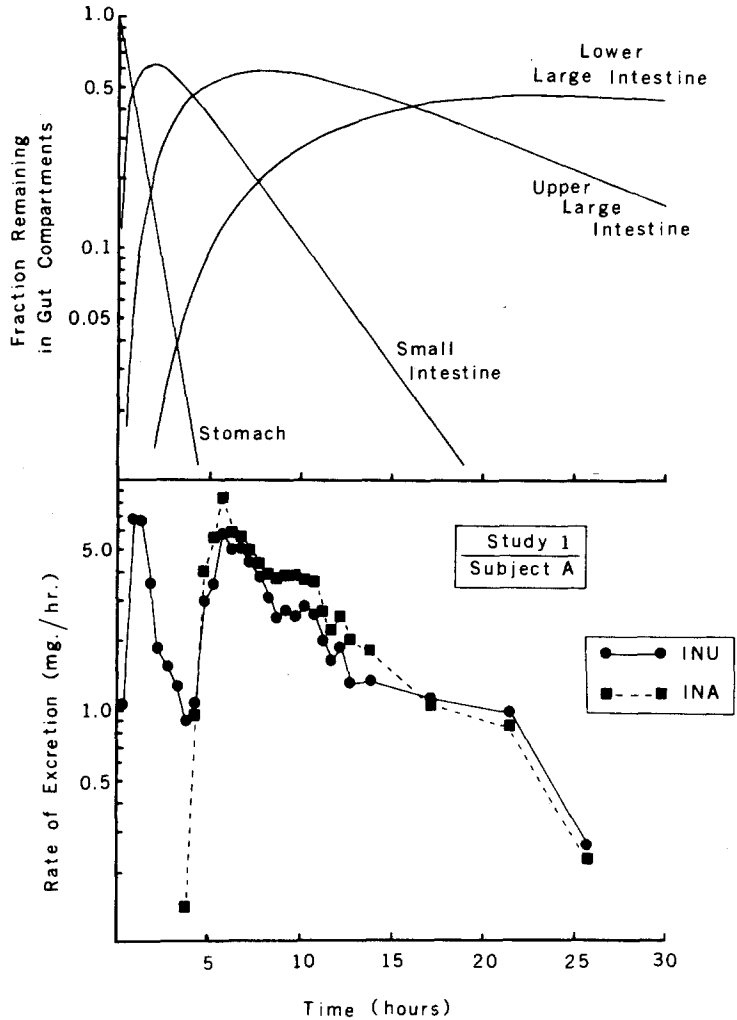

Fig. 11. Relationship between rates of excretion of isonicotinuric and isonicotinic acids following oral administration of isonicotinuric acid to subject A and fraction of food residues in gastrointestinal compartments.

various segments of the gastrointestinal tract; also shown are the INU and INA excretion rate curves from study 1. It is quite clear that at the times that INA was being absorbed, much of the ingested INU would have passed into the large intestine. Since the model probably underestimates gastrointestinal transit in the present studies, it is indeed likely that more INU was present in the large intestine at the critical times than would be predicted by the model.

Another argument supporting the hypothesis that INU hydrolysis occurred within the large intestine arises from an examination of the microbial populations of the normal human gut. Scheline (2) reviewed much of the literature on the distribution of microorganisms in the gastrointestinal tract and indicated that the stomach, duodenum, jejunum, and upper ileum are only sparsely populated. Increasing numbers of organisms exist in the distal ileum, and a significant increase is seen at the ileocecal valve. The numbers and distribution of organisms are shown in Fig. 12, which is redrawn from Broitman and Giannella (36). Abrupt increases in microbial populations occur in the cecum. Thus it appears likely that most of the bacterial hydrolysis of INU occurred distal to the ileocecal valve.

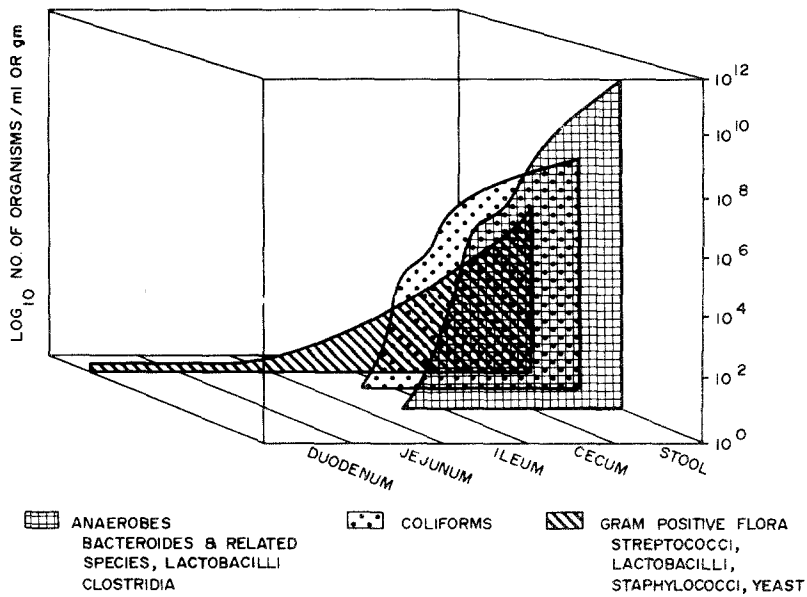

Fig. 12. Diagramatic scheme of microbial populations of the gastrointestinal tract. Redrawn from Broitman and Giannella (36) with permission of copyright owner and S. A. Broitman.

Absorption of Intact Isonicotinuric Acid and Possible Dose-Dependent Kinetics of Isonicotinic Acid

Some of the amounts of INU absorbed intact have been mentioned previously, but the method by which these calculations were made was not discussed. For the purpose of this calculation, it was assumed that absorption of intact INU ceased when either (a) the initial INU rate of excretion curve showed a very definite upswing or (b) the INA rate of excretion exceeded the INU rate of excretion. While these two criteria are somewhat arbitrary, only small errors result, as a consequence of most of the intact INU having been absorbed prior to appearance of urinary INA. The one possible exception was study 10, in which INU was administered rectally; here the error could be considerably greater. The Loo–Riegelman equation (31) was used to calculate the cumulative amount of INU absorbed. The percent metabolism of INA to INU was then readily calculated from cumulative urinary excretion data. Compartment model parameters were available for subjects A and B, whereas the average parameter values for these subjects were used for subject C. The results from the various studies are summarized in Table II.

In study 4, when 476 mg INU was administered to subject A, 28.5% of the absorbed INA was conjugated with glycine. In contrast, when lower doses were administered in studies 1, 7, 8, and 10, the average percent conjugation of INA with glycine was 41.35. It appears that a larger amount of INA in the body may have partially saturated the glycine conjugation system. Similar observations have been reported by Peters *et al.* (58) and Ellard and Gammon (30).

Application of the Loo–Riegelman equation for drug absorption requires that parameters obtained following intravenous drug administration be operative following oral administration. It was because the percent conjugation of INA with glycine for subject A was similar in the i.v. study and in study 4 that the Loo–Riegelman equation was applied to study 4 (Fig. 5). Additionally, the i.v. studies of INA and INU from which the disposition parameters for the Loo–Riegelman equation were obtained were conducted with controlled urinary *p*H, whereas most of the studies on gut metabolism were conducted without urinary *p*H control. No doubt this introduced an error in the calculations, but this is probably not significant because of the rapid renal clearance of INA and INU. It has been observed that the INA excretion rate is somewhat dependent on urinary *p*H (43). Nonetheless, reported INA half-lives do not differ significantly in studies with and without urinary *p*H control. For example, Ellard and Gammon (30) reported a half-life for INA of approximately 0.7 hr when either 25 or 250 mg INA was administered orally without urinary *p*H control. In the

Table II. Summary of Data from the Various Studies

Study	Subject	Compound administered	Dose and route of administration	Adjuvant	Percent of dose recovered in urine	Percent of dose absorbed as intact INU	Percent of INA metabolized to INU
1	A	INU	129 mg oral	None	77.9	9.44	45.6
2	B	INU	129 mg oral	None	69.1	3.93	49.2
3	C	INU	130 mg oral	None	68.8	9.12	50.3
4	A	INU	476 mg oral	None	62.7	7.20	28.5
5	A	INU	129 mg oral	$NaHCO_3$	91.9	4.70	44.0
6	A	INU	129 mg oral	$NaHCO_3$	67.9	10.3	37.0
7	A	INU	130 mg oral	Propantheline	48.1	28.6	37.2
8	A	INU	128 mg oral	Kanamycin	69.4	13.7	44.6
9	A	INU	124 mg oral	Lincomycin	5.95	5.95	—
10	A	INU	49.0 mg rectal	None	97.2	5.57	38.0
—	A	INA	200 mg i.v. infusion	$NaHCO_3$	100	—	24.3
—	B	INA	197 mg i.v. infusion	$NaHCO_3$	100	—	26.2

i.v. studies of Boxenbaum and Riegelman (21), approximately 200 mg INA was infused intravenously for 1 hr and urinary *p*H was maintained at 8.0 ± 0.2; terminal exponential half-life (β-phase) was approximately 0.76 hr.

Gastrointestinal Metabolism as a Factor in Drug Activity

From the foregoing discussion, it is apparent that gastrointestinal microflora may have a profound effect on an orally administered compound. For example, drug dosage forms exhibiting poor bioavailability characteristics in the upper gastrointestinal tract enhance the likelihood of bacterial metabolism in the lower gastrointestinal tract.

Bacterial metabolism, however, may be a desirable property. Salicylazosulfapyridine is reduced by intestinal bacteria to 5-aminosalicylic acid and sulfapyridine (59); the beneficial effect of this drug in ulcerative colitis may well be due to the delivery of these metabolites to the site in the colon where either or both exert their effect.

Conversely, bacterial metabolic products may be responsible for drug toxicity. In this respect, Holt (60) presented an interesting hypothesis. He noted that there seems to be no reported case of aplastic anemia resulting from administration of chloramphenicol solely by the parenteral route. The toxicity of this antibiotic when administered by the oral route might be attributed to unusually large populations of gut enterobacteria capable of degrading the chloramphenicol to toxic by-products. It was estimated that fewer than 2% of the children studied had a gut flora of enterobacteria capable of significant chloramphenicol metabolism.

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