yielded 137 mg of IV as a colorless oil; IR (film): 3310 (N–H), 3050, 3020, 1600, 750, 700 (aromatic), 2910, 2810 (C–H), and 1635 (C=O) cm^{-1}; NMR ($CDCl_3$): δ 1.34 (s, 1, N–H, exchangeable), 2.66–2.94 (m, 2, C–CH_2–NH), 3.25–3.78 [m, 4, C–CH_2–N–C(–)=O and NH–CH_2-phenyl], 4.66 (s, 2, phenyl-CH_2–N–C=O), and 7.13–7.50 (m, 15, aromatic).

Benzoylation of Benzathine to Give IV—To 960 mg of I in 3 ml of chloroform and 0.5 ml of triethylamine was added dropwise 550 mg of benzoyl chloride in 1 ml of chloroform. After 1.5 hr at room temperature, the solution was added to 20 ml of water, brought to pH 11 with sodium hydroxide, and extracted with chloroform. Workup afforded 1.5 g of white solid. A portion of the crude product was chromatographed by preparative TLC to yield 65 mg of IV.

1,3-Dibenzyl-2-phenyltetrahydroimidazole (V)—Equimolar (0.02 *M*) amounts of I and benzaldehyde with 150 mg of *p*-toluenesulfonic acid in 100 ml of benzene were condensed over 3 hr to yield a white solid, 4.1 g, mp 99–99.5° (hexane) [lit. (5) mp 99°].

Oxidation of V to II—To 1.235 g (3.76 mmoles) of V in 10 ml of benzene was added dropwise 956 mg (3.76 mmoles) of iodine in 100 ml of benzene. To the resulting solution was added, all at once, an equal aliquot of iodine in benzene. The solution was cooled, and the red solid, 2.8 g (90%), was collected. Recrystallization from methanol–acetone gave deep-red needles of II, mp 163.5–166°.

RESULTS AND DISCUSSION

The reaction of benzathine with iodine in methanol resulted in the isolation of one product; the NMR spectrum was consistent with Structure II. Further confirmation of Structure II was derived from the partial reduction of II to the iodide III, which, under basic conditions, yielded the ring-opened benzoylbenzathine (IV), analogous to other imidazolinium salts (6).

Unambiguous confirmation of Structure II resulted from the iodine oxidation of the tetrahydroimidazole (V) to II.

Product II probably results from a reaction sequence beginning with oxidation of benzathine to give an imine, which then hydrolyzes to give the benzaldehyde (Scheme I). The benzaldehyde is free to condense with intact benzathine to give V, which oxidizes with excess iodine to II.

Therefore, this reaction sequence leads to erroneously low results in the CFR iodometric assay as presently described for penicillin V benzathine. It also accounts for the fact that interference by benzathine in the iodometric assay is dependent on pH since the initial oxidation requires a free amino function.

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Capacity-Limited Gut Wall Metabolism of 5-Aminosalicylic Acid, a Therapeutically Active Metabolite of Sulfasalazine, in Rats

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Abstract □ The metabolic fate of 5-aminosalicylic acid (reported to be the active therapeutic moiety of sulfasalazine) was assessed in fasting rats as a function of dose (25–200 mg/kg) and administration route (oral, intraperitoneal, and intravenous). 5-Aminosalicylic acid is subject to both capacity-limited presystemic (apparently during first passage through the intestinal epithelium) and systemic acetylation. The possibility exists that 5-aminosalicylic acid also is acetylated presystemically after oral sulfasalazine administration to patients with inflammatory bowel disease. Any alteration in the absorption rate of this active metabolite from the colon could affect the time course of local anti-inflammatory activity if *N*-acetyl-5-aminosalicylic acid is inactive or less active than 5-aminosalicylic acid.

Keyphrases □ 5-Aminosalicylic acid—metabolism, gut wall, effect of dose and administration route □ Sulfasalazine metabolites—5-aminosalicylic acid, metabolism, gut wall, effect of dose and administration route □ Antibacterial agents—sulfasalazine, 5-aminosalicylic acid metabolite, metabolism, gut wall, effect of dose and administration route □ Intestinal epithelium—metabolism, 5-aminosalicylic acid, effect of dose and administration route

Sulfasalazine (salicylazosulfapyridine) is widely used for the treatment of ulcerative colitis and Crohn's disease (1). After oral administration to rats and humans, the drug is almost completely metabolized in the colon and cecum by bacterial azo reductases. The metabolic products sulfapyridine and 5-aminosalicylic acid are absorbed from the colon and are further metabolized to N^4-acetylsulfapyridine, to the *O*-glucuronide and *O*-sulfate conjugates of 5′-hydroxysulfapyridine and N^4-acetyl-5′-hydroxysulfapyridine, and to *N*-acetyl-5-aminosalicylic acid (2–6).

Several investigators (3, 5–8) suggested that sulfasalazine itself may not be an active therapeutic agent but may serve only as a means of delivering its metabolic products, 5-aminosalicylic acid (a possible anti-inflammatory agent) and sulfapyridine (an antibacterial agent), to the inflammation site in the colon where either or both of these agents exert the desired pharmacological effects. Recent studies in which sulfasalazine, 5-aminosalicylic acid, and sulfapyridine were administered rectally to patients with ulcerative colitis (9) and idiopathic proctitis (10) suggest that 5-aminosalicylic acid is the active therapeutic moiety of sulfasalazine and acts topically on the inflammed mucosa. Although this finding requires confirmation in a larger patient population and does not explain the mode of action of 5-aminosalicylic acid, it may be relevant that sulfasalazine and 5-aminosalicylic acid are inhibitors of prostaglandin synthesis (10–13) and that patients with active ulcerative colitis have increased fecal levels and colonic venous blood levels of prostaglandins (13). Prostaglandins are known to be involved in inflammation

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Table I—Effect of Dose and Administration Route on 5-Aminosalicylic Acid Biotransformation in Fasting Rats

Administration Route	Dose of 5-Aminosalicylic Acid[a], mg/kg	Mean Urinary Excretion in 48 hr ($n = 4$) ± SE		
		Total Recovery, % of dose	N-Acetyl-5-aminosalicylic Acid, % of total[b]	Free 5-Aminosalicylic Acid, % of total[b]
Oral	25	90.5 ± 3.8	82.0 ± 2.8	18.0 ± 2.8
	50	91.0 ± 5.7	69.8 ± 4.3	30.2 ± 4.3
	100	95.2 ± 7.1	50.1 ± 5.5	49.9 ± 5.5
	200	101 ± 3.3	33.2 ± 2.4	66.8 ± 2.4
Intraperitoneal	25	101 ± 2.4	54.2 ± 4.3	45.8 ± 4.3
	50	104 ± 1.7	41.3 ± 3.0	58.7 ± 3.0
	200	104 ± 3.3	29.8 ± 5.9	70.2 ± 5.9
Intravenous	25	102 ± 2.1	55.8 ± 6.4	44.2 ± 6.4
	50	92.3 ± 1.9	44.8 ± 4.8	55.2 ± 4.8
	200	103 ± 2.4	25.9 ± 5.2	74.1 ± 5.2

[a] Administered as an aqueous solution (pH 7). [b] Free and acetylated 5-aminosalicylic acid.

(10–13). No information exists in the literature related to factors affecting the biotransformation of 5-aminosalicylic acid.

The present study used the rat as an animal model to assess whether 5-aminosalicylic acid is acetylated presystemically after oral administration and to explore the possible dose-dependent characteristics of this metabolic process.

EXPERIMENTAL

Reagents—5-Aminosalicylic acid[1] was purified prior to use (14). Reagent grade sodium nitrite[2], ammonium sulfamate[2], N-(1-naphthyl)-ethylenediamine dihydrochloride[2], acetic anhydride[2], and isopropylacetone[2] (methyl isobutylketone) were used as received.

Animals—Adult male Sprague–Dawley rats[3], 250–350 g, were fasted for 16 hr prior to the studies but had access to water at all times. Food[4] was supplied 24 hr after drug administration. All biotransformation studies were initiated between 9:00 and 10:00 am to eliminate possible circadian variation.

Effect of Dose and Administration Route on 5-Aminosalicylic Acid Metabolism—The same four fasting rats received single doses of 5-aminosalicylic acid (in aqueous solution at pH 7), 25–200 mg/kg po, ip, or iv, in two four-way and one two-way crossover studies. In a subsequent study, 8.33 mg of 5-aminosalicylic acid/kg was administered intraperitoneally to the fasting animals every 20 min for three doses. Dosing volumes of 5.0 and 2.5 ml/kg were used for oral and parenteral drug administrations, respectively.

Quantitative urine collections were made daily for 2 days, and the specimens were stored at −20° pending assay for free and N-acetylated 5-aminosalicylic acid. A 4-day washout period was allowed between drug administrations.

Assay of Free and Acetylated 5-Aminosalicylic Acid in Urine—A specific colorimetric method (15) was employed. Accordingly, 1 ml of urine (previously centrifuged and diluted with distilled water when necessary) was acidified with 1.0 ml of 1.0 M HCl, and N-acetyl-5-aminosalicylic acid was first extracted directly into 5.0 ml of isopropylacetone and then reextracted from a 4.0-ml aliquot of the organic (upper) phase into 3.0 ml of pH 6.0 phosphate buffer. After centrifugation, a 0.5-ml aliquot of the buffered aqueous phase was withdrawn, mixed with 0.5 ml of 8.0 M HCl in a polytef-lined screw-capped culture tube, and heated at 100° for 45 min to effect metabolite deacetylation.

The acidic solution was cooled to room temperature, and the free 5-aminosalicylic acid was subjected to a modified Bratton–Marshall reaction (16) by adding, at 3-min intervals, 1.0-ml quantities of aqueous solutions of sodium nitrite (0.12%), ammonium sulfamate (0.8%), and N-(1-naphthyl)ethylenediamine dihydrochloride (0.8%). The reaction mixture was stored in the dark for 4 hr to allow for maximum color development, and the absorbance of the violet-colored solution was measured on a double-beam spectrophotometer[5] at 560 nm using 1.0-cm cells.

Total 5-aminosalicylic acid (free and acetylated) was analyzed by the same procedure after quantitative acetylation of the free 5-aminosalicylic acid content of a second biological sample with 20 μl of acetic anhydride. The amount of free 5-aminosalicylic acid present in the urine specimen was determined from the difference between the total and acetylated 5-aminosalicylic acid assay results. A linear Beer's law calibration plot was constructed by subjecting standard samples containing 0–70 μg of 5-aminosalicylic acid/ml to the procedure for total 5-aminosalicylic acid.

Twenty-four-hour urine blank values were small but were used to correct all free and acetylated 5-aminosalicylic acid excretion data.

Statistical Analysis—Differences among more than two mean values were evaluated by analysis of variance, and differences between any two means were compared using Tukey's multiple comparison test (at $p = 0.05$) or the Student paired t test (17, 18).

RESULTS AND DISCUSSION

The results of the study of 5-aminosalicylic acid biotransformation after intraperitoneal, intravenous, and oral administration of 25–200-mg/kg doses to the same group of four fasting rats are presented in Table I. Analyses of variance indicated that the urinary recovery of total 5-aminosalicylic acid (sum of free and acetylated drug) was independent of dose and administration route ($F_{9,30} = 2.17$, $p > 0.05$). Recovery ranged from 91 to 104% of the dose, with 90–95% of the total recovery being achieved within 24 hr after drug administration.

The mean fraction of total 5-aminosalicylic acid excreted in the urine as the N-acetylated metabolite significantly decreased as the intraperitoneal and intravenous doses of 5-aminosalicylic acid were increased from 25 to 200 mg/kg ($F_{2,9} = 7.24$ for the intraperitoneal route and $F_{2,9} = 7.58$ for the intravenous route, $p < 0.025$; Table I). Lukas *et al.* (19) demonstrated that drugs administered intraperitoneally are absorbed primarily into the portal circulation and, therefore, must pass through the liver before reaching the systemic circulation. After intravenous administration, only 28% of the cardiac output reaches the liver (19). Thus, the fact that no significant difference in the degree of acetylation existed between the two routes after each dose ($p > 0.05$; Table I) suggests that 5-aminosalicylic acid is not subject to first-pass liver metabolism and that its systemic elimination is capacity limited. The lack of an effect of the intraperitoneal administration rate on drug biotransformation after the 25-mg/kg dose (Table II) suggests that systemic 5-aminosalicylic acid elimination is linear at or below this dosage level.

Dose-dependent decreases in the extent of 5-aminosalicylic acid metabolism were also observed after oral administration ($F_{3,12} = 10.8$, $p < 0.001$; Table I). The drug fraction converted to the acetylated metabolite after the 25- and 50-mg/kg po doses were greater than those observed after comparable intraperitoneal and intravenous doses ($p < 0.05$, Tukey's multiple range tests; Table I). These findings suggest that 5-aminosalicylic acid is subject to capacity-limited presystemic (gut wall) and systemic acetylation after oral administration. Based on the average metabolic data presented in Table I, the extent of acetylation by the intestinal epithelium was estimated to be ~25–28% of a 25- or 50-mg/kg po dose. The N-acetyltransferase enzyme systems at the presystemic site appear to be nearly saturated after a 200-mg 5-aminosalicylic acid/kg dose ($F_{2,9} = 0.579$, $p > 0.1$; Table I).

The ability of enzyme systems present in the intestinal epithelium to acetylate the structurally related drugs p-aminobenzoic acid and p-aminosalicylic acid *in vitro* is well documented (20, 21). p-Aminobenzoic acid (22) and p-aminosalicylic acid (23, 24) are also subject to capacity-

[1] Aldrich Chemical Co., Milwaukee, Wis.
[2] Fisher Scientific Co., Rochester, N.Y.
[3] Blue Spruce Farms, Altamont, N.Y.
[4] Charles River rat chow.
[5] Beckman DB-G, Beckman Instruments, Fullerton, Calif.

Table II—Effect of Administration Rate on 5-Aminosalicylic Acid Biotransformation in Fasting Rats

Rat	Urinary Excretion in 48 hr: Total Recovery, % of dose		*N*-Acetyl-5-aminosalicylic Acid, % of total[a]	
	Regimen A[b]	Regimen B[c]	Regimen A[b]	Regimen B[c]
1	99.3	104	58.2	53.8
2	104	103	63.4	57.4
3	105	98.2	43.7	42.8
4	94.4	99.0	51.6	51.1
Mean	101	101	54.2	51.3
SE	2.4	1.4	4.3	3.1
Paired *t* test	N.S. ($p > 0.8$)		N.S. ($p > 0.1$)	

[a] Free and acetylated 5-aminosalicylic acid. [b] Regimen A = single 25 mg/kg ip dose. [c] Regimen B = 8.33 mg/kg ip every 20 min for three doses.

limited acetylation during their intestinal absorption in humans. Studies are currently in progress to determine whether a food-induced decrease in the gastric emptying rate can, by decreasing the rate of 5-aminosalicylic acid absorption, further increase the extent of gut wall metabolism.

These results demonstrate that orally administered 5-aminosalicylic acid is subject to both capacity-limited gut wall and systemic metabolism. A similar capacity-limited presystemic metabolic profile for 5-aminosalicylic acid may exist after oral administration of sulfasalazine to patients with ulcerative colitis and Crohn's disease. If *N*-acetyl-5-aminosalicylic acid is inactive or less active than 5-aminosalicylic acid (a possibility remaining to be explored), then the time course of local anti-inflammatory activity may be affected.

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4-Acetoxy-1,2,3,4-tetrahydro-2,2-dimethyl-6,7-methylenedioxyisoquinolinium Iodide, an Acetylcholine Analog

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Abstract □ 4-Acetoxy - 1,2,3,4 - tetrahydro - 2,2-dimethyl-6,7-methylenedioxyisoquinolinium iodide, an analog of acetylcholine, was synthesized and a pharmacological profile of its GI effects was compiled. The agent inhibited dog colonic contraction in response to pelvic nerve stimulation and to acetylcholine. In rats, the compound markedly reduced gastric acid output and the volume of gastric secretions. The lack of inhibition of chromodacryorrhea production in response to carbachol indicates a lack of anticholinergic action. The agent failed to affect the acute inflammatory response of the rat hindpaw in response to carrageenan. The precursor of the compound was ineffective in the pharmacological tests.

Keyphrases □ Acetylcholine analogs—synthesis, pharmacodynamics, structure–activity relationships □ Cholinergic agents—acetylcholine analogs, synthesis, pharmacodynamics, structure–activity relationships □ Structure–activity relationships—acetylcholine analogs

As part of a program directed toward the synthesis of novel GI agents, an acetylcholine analog was desired in which the tetraalkylammonium function of the molecule was incorporated as part of a rigid annular system. Accordingly, a route to 4-acetoxy-1,2,3,4-tetrahydro-2,2-dimethyl-6,7-methylenedioxyisoquinolinium iodide (I) was devised.

DISCUSSION

Reaction of the previously reported (1) 4-hydroxy-6,7-methylenedioxy-1,2,3,4-tetrahydroisoquinoline (II) with methyl iodide gave the quaternary Compound III which, upon treatment with acetic anhydride, afforded the racemic acetylcholine analog I.

Compound I, 10 mg/kg iv in three dogs, caused marked, but brief (20 min), antagonism of colonic contractions in response to intermittent pelvic nerve stimulation (2). In addition, acetylcholine-induced con-

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