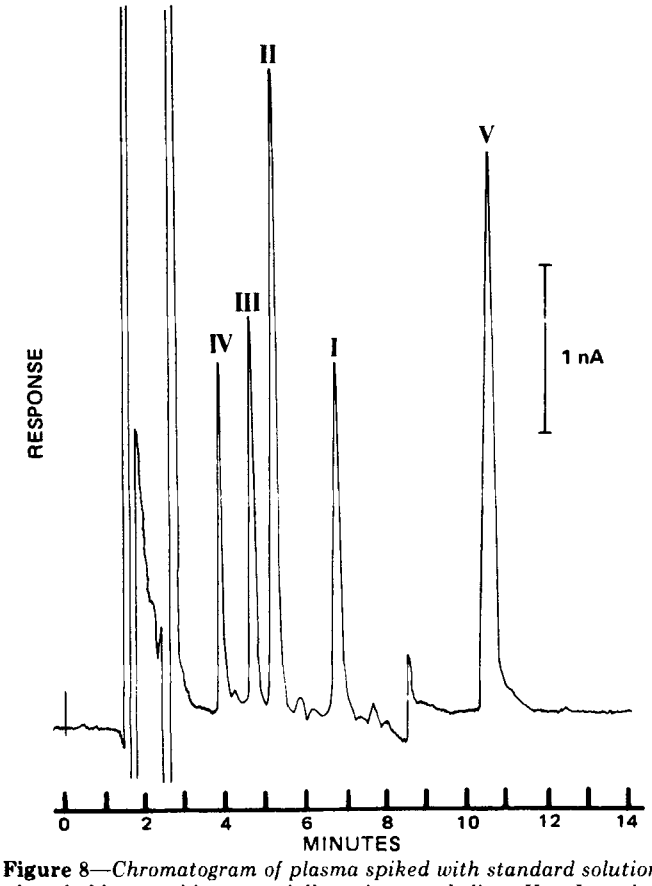

Figure 8—*Chromatogram of plasma spiked with standard solutions of methyldopa and its potentially active metabolites. Key: I, methyldopa; II, 3,4-dihydroxybenzylamine; III, methylnorepinephrine; IV, norepinephrine; and V, methyldopamine.*

Dopamine eluted with methyldopa with a retention time of 6.9 min. However, dopamine does not compromise the assay of methyldopa since normal plasma dopamine concentrations are below the sensitivity of this assay. Blank plasma samples showed no detectable interference. Changing the mobile phase to 9% acetonitrile and pH 2.40 and the ion-pair to 5 m*M* pentanesulfonic acid (sodium salt), with all other parameters remaining constant, allows complete resolution of methyldopa from dopamine. Authentic plasma samples of subjects receiving methyldopa also showed no detectable dopamine in the presence of methyldopa when resolved.

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Interactions of Aspirin with Acetaminophen and Caffeine in Rat Stomach: Pharmacokinetics of Absorption and Accumulation in Gastric Mucosa

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Received December 3, 1979, from the **National Institute of Public Health, P.O. Box 1, 3720 BA Bilthoven, The Netherlands*, and the ‡*Department of Pharmacology, Faculty of Pharmacy, State University of Utrecht, Utrecht, The Netherlands.* Accepted for publication February 27, 1980.

Abstract □ To study the pharmacokinetic interactions between aspirin (250 mg/kg) and simultaneously administered oral acetaminophen (125 mg/kg) or caffeine (50 mg/kg) in male adult rats, noninterfering GLC assays for these drugs were developed. Acetaminophen and caffeine both retarded the appearance of salicylate in plasma. During the elimination phase, acetaminophen enhanced plasma salicylate levels whereas caffeine did not. Aspirin reduced the plasma levels of both acetaminophen and caffeine during absorption and elimination. Regardless of whether the drugs had been administered separately or in combination, higher concentrations of salicylate, acetaminophen, and caffeine were found in the glandular part of the stomach compared to the nonglandular part (rumen). In both parts, the absorption of acetaminophen increased in the presence of aspirin. Simultaneous administration of aspirin with caffeine did not influence the absorption of either drug in the glandular and ruminal parts. The inhibitory action of acetaminophen and the potentiating action of caffeine on the erosive activity of aspirin are not due to any effects of these drugs on salicylate accumulation in glandular tissue.

Keyphrases □ Aspirin—administered alone and with acetaminophen or caffeine, absorption, distribution in gastric tissue, blood plasma levels, rats □ Acetaminophen—administered alone and with aspirin, absorption, distribution in gastric tissue, blood plasma levels, rats □ Caffeine—administered alone and with aspirin, absorption, distribution in gastric tissue, blood plasma levels, rats □ Pharmacokinetics—aspirin administered alone and with acetaminophen or caffeine, absorption, distribution in gastric tissue, blood plasma levels, rats

Irritation of the GI mucosa, production of erosions, and GI bleeding are serious side effects of aspirin and other nonsteroidal anti-inflammatory drugs (1, 2). Acidic anti-inflammatory drugs are thought to cause these side effects

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following their rapid absorption to give very high intracellular concentrations, finally resulting in cell death (3–5). However, a causal relationship between the intracellular accumulation of salicylate and the decay of gastric mucosal cells has not been proven. Therefore, the present study was initiated to compare the characteristics of absorption and distribution of aspirin in the rat stomach with those of acetaminophen and caffeine, two drugs that are nonulcerogenic in rats (6).

It also was suggested (5) that the reduced gastric toxicity of slow-release and enteric-coated aspirin formulations (7–9) might be explained by the delayed absorption of aspirin and, thus, a reduction in salicylate trapping in parietal cells. In the rat, acetaminophen reduced the erosive activity of aspirin whereas caffeine potentiated this activity (6, 10). To test this hypothesis, the effects of acetaminophen and caffeine on the absorption of salicylate into the cells of the glandular (containing the parietal cells) and nonglandular parts of the rat stomach were investigated.

EXPERIMENTAL

Reagents and Materials—Aspirin[1], salicylic acid[1], acetaminophen[1], caffeine[1], phenacetin[1], and polysorbate 80[2] were obtained commercially. All other chemicals were analytical reagent grade. Ether[3] was distilled every day. Male Wistar rats of an inbred strain, 180–200 g, were purchased locally[4].

GLC—For aspirin and salicylic acid, a gas chromatograph[5] equipped with a multitemperature programmer, a flame-ionization detector, and an integrator[6] was used. The glass column, 1.00 m × 3 mm i.d., was packed with 3% OV-17[7] on 80–100-mesh Gas Chrom Q[7]. The column oven temperature was programmed for 2 min at 105°, an increase of 4°/min from 105 to 130°, an increase of 30°/min from 130 to 195°, 2 min at 195°, and then cooling down. The injector temperature was 220°, the detector temperature was 220°, the argon carrier gas flow rate was 35 ml/min, the hydrogen flow rate was 40 ml/min, the air flow rate was 400 ml/min, and the amplifier range was at 10. Under these conditions, the retention times of the trimethylsilyl ether derivatives were 3.0 min for *p*-toluic acid (internal standard), 5.0 min for salicylic acid, and 7.5 min for aspirin.

For acetaminophen, a gas chromatograph[8] equipped with a flame-ionization detector and a computing integrator[9] was used. The glass column, 1.80 m × 2 mm i.d., was packed with 3% OV-101[7] on 80–100-mesh Gas Chrom Q. The column oven temperature was 135°, the injector temperature was 200°, the detector temperature was 220°, the nitrogen carrier gas flow rate was 30 ml/min, the hydrogen flow rate was 35 ml/min, and the air flow rate was 350 ml/min. The retention times of the trimethylsilyl ether derivatives were 4.5 min for *p*-bromoacetanilide (internal standard), 7.5 min for acetaminophen, and 6.0 min for phenacetin.

For caffeine, a gas chromatograph[8] equipped with an alkali flame-ionization detector and a computing integrator[9] was used. The glass column, 1.50 × 2 mm i.d., was packed with 3% OV-101 on 80–100-mesh Gas Chrom Q. The column oven temperature was 165°, the injector temperature was 220°, the detector temperature was 220°, the nitrogen carrier gas flow rate was 30 ml/min, the hydrogen flow rate was 35 ml/min, and the air flow rate was 300 ml/min. Under these conditions, the retention times were 5.0 min for phenacetin (internal standard) and 8.0 min for caffeine.

Drug Treatment and Sample Collection—Rats were distributed randomly into groups of eight or 10 animals and were deprived of food, but not water, for 36 hr. They were kept in cages (0.32 × 0.20 × 0.20 m) with a metal grid (8 × 8 mm) to avoid coprophagy. Room temperature was maintained at 21 ± 0.5°. Aspirin, acetaminophen, and caffeine were ground before use (<110 μm). All treatments were administered orally at 5 ml/kg as suspensions in 4% polysorbate 80; control rats received a similar volume of 4% polysorbate 80 solution.

Rats were treated with aspirin (250 mg/kg), acetaminophen (125 mg/kg), caffeine (50 mg/kg), aspirin and acetaminophen (250 and 125 mg/kg), and aspirin and caffeine (250 and 50 mg/kg). Blood samples (1.0 ml) were obtained by decapitation. In the drug elimination studies, dosage groups of 10 rats were decapitated at 0.5, 1.5, 4, 8, and 17 hr after administration. In the drug absorption studies, dosage groups of eight rats were decapitated at 5, 10, 15, 20, and 30 min after administration.

In the absorption experiments, the stomachs were removed immediately after blood sampling, opened along the greater curvature, and rinsed in saline. The glandular part of the gastric tissue was scraped off, and the ruminal part was cut off. Blood was collected in polyethylene snap caps, which contained 1 drop of a solution of 250 IU of heparin[10] and 12 mmoles of sodium fluoride/ml. After centrifugation[11] at 1000×*g*, the plasma was stored at −20°. Tissue samples were weighed wet, stored at −20°, and homogenized[12] in water (1:3 w/v) just before analysis.

Assay of Aspirin and Salicylic Acid—To a 15-ml stoppered conical glass centrifuge tube were added 0.1–0.5 ml of plasma or 1 ml of tissue homogenate, 200–300 mg of sodium chloride, 0.5 ml of buffer solution (17.2 mmoles of potassium sulfate in 100 ml of 0.2 *M* H_2SO_4), and 10 ml of ether. After shaking[13] for 30 min and centrifuging for 10 min at 1000×*g*, as much ether as possible was transferred into a conical tube and evaporated to dryness on a heating block[14] at 30° under a nitrogen stream. To the dry residue was added 0.1 ml of 0.6 m*M* *p*-toluic acid in acetone.

The contents of the tube were transferred with four 0.1-ml portions of acetone to a micro reaction vessel[7]. After evaporation of the acetone, the residue was dissolved in 50 μl of bis(trimethylsilyl)trifluoroacetamide[7], and the vessel was closed with a polytetrafluoroethylene-lined rubber septum[7]. After agitation and heating at 50° for 1 hr, 1 μl was injected into the GLC column and the temperature programming was started.

Assay of Acetaminophen—To a 10-ml glass-stoppered centrifuge tube were added 0.1–1 ml of plasma or 1 ml of tissue homogenate, 2 ml of buffer solution (1 *M* dibasic potassium phosphate saturated with sodium chloride and brought to pH 7.4 with 1 *M* NaOH), and 5 ml of ether containing 24 nmoles of *p*-bromoacetanilide. After shaking for 30 min and centrifuging for 10 min at 1000×*g*, the organic layer was transferred to a conical glass tube and evaporated under a nitrogen stream at room temperature.

The residue was transferred to a micro reaction vessel with two 150-μl portions of acetone; after evaporation of the acetone, the residue was dissolved in 50 μl of bis(trimethylsilyl)trifluoroacetamide and the vessel was closed with a polytetrafluoroethylene-lined rubber septum. After agitation and heating at 50° for 2 hr, 1 μl was injected into the GLC column.

Assay of Caffeine—To a 10-ml glass-stoppered centrifuge tube was added 0.1 ml of a solution of 0.1 m*M* phenacetin in acetone. After evaporation of the acetone, 0.1–0.5 ml of plasma or 1 ml of tissue homogenate and 5 ml of dichloromethane were added. After shaking for 15 min and centrifuging for 5 min at 1000×*g*, the organic layer was dried over sodium sulfate and evaporated to dryness at 40° under a nitrogen stream. The residue was dissolved in 100 μl of methanol, and 1 μl was injected into the GLC column.

Data Analysis—The means and standard errors of the mean (*SEM*) of plasma and tissue concentrations of total salicylate (acetylsalicylate and salicylate), acetaminophen, and caffeine are presented in Figs. 1–4 (the standard error of the mean is presented only when it exceeds the dimensions of the symbol). Rankit tests (11) showed that the assumption that the concentrations within treatment groups were normally distributed was justified.

Differences between the mean concentrations of the treatment groups were tested using the Student *t* test. Because the ratio of two normally distributed variables is not normally distributed, differences in concentration ratios between treatment groups were tested using Wilcoxon's signed rank test. Differences were assumed to be real when tests indicated probability levels of <5%.

[1] O. P. G., Utrecht, The Netherlands.
[2] Brocacef B. V., Maarssen, The Netherlands.
[3] Merck, Darmstadt, West Germany.
[4] Centraal Proefdierenbedrijf T. N. O., Zeist, The Netherlands.
[5] F 22, Perkin-Elmer and Co., GMBH, Uberlingen, West Germany.
[6] Spectra-Physics, Santa Clara, Calif.
[7] Supelco Inc., Bellefonte, Pa.
[8] Model 1440, Varian Aerograph, Walnut Creek, Calif.
[9] CDS 111 C, Varian, Palo Alto, Calif.
[10] Leo, Ballerup, Denmark.
[11] Model GLC-1 centrifuge, Sorvall, Newtown, Conn.
[12] Model S 45 homogenizer, Virtis Co., Gardiner, N.Y.
[13] Griffin flask shaker, Griffin and George Ltd., London, England.
[14] Dri-block DB-3, Techne, Princeton, N.J.

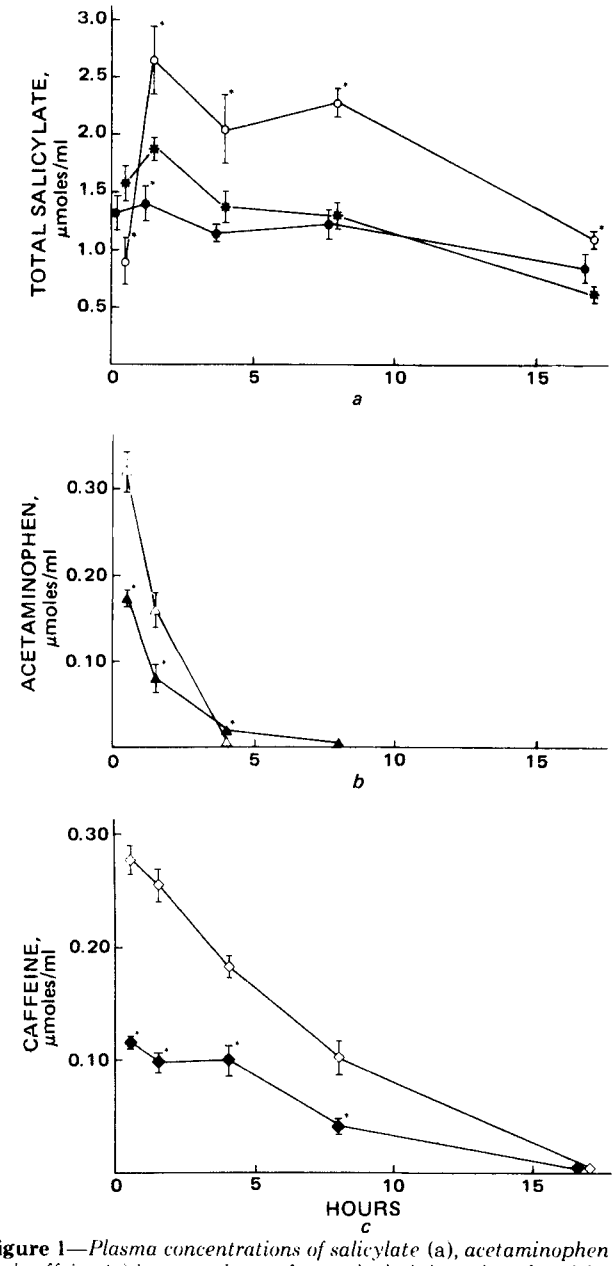

Figure 1—*Plasma concentrations of salicylate* (a), *acetaminophen* (b), *and caffeine* (c) *in starved rats after oral administration of aspirin (250 mg/kg)* (*), *acetaminophen (125 mg/kg)* (△), *caffeine (50 mg/kg)* (◇), *aspirin* (○) *and acetaminophen* (▲) *(250 and 125 mg/kg, respectively), and aspirin* (●) *and caffeine* (♦) *(250 and 50 mg/kg, respectively). Values are means* ± SEM *from 10 rats. An asterisk indicates* $p < 0.05$ *compared to the drugs given alone.*

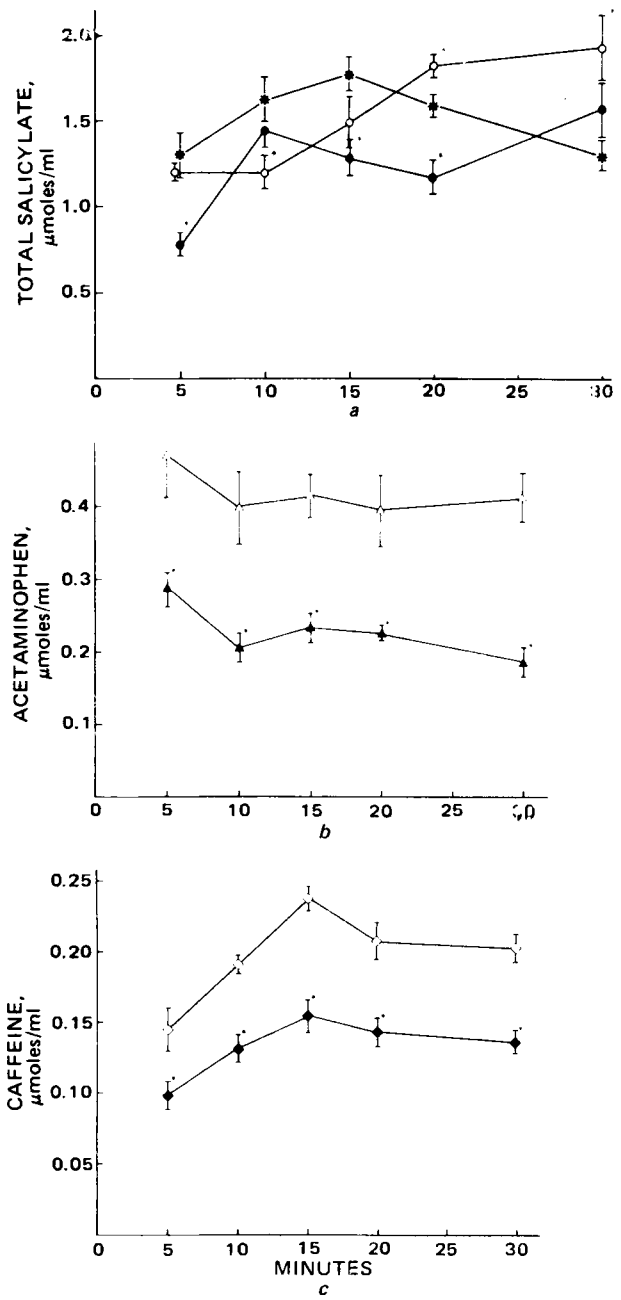

Figure 2—*Plasma concentrations during absorption of salicylate* (a), *acetaminophen* (b), *and caffeine* (c) *in starved rats after oral administration of aspirin (250 mg/kg)* (*), *acetaminophen (125 mg/kg)* (△), *caffeine (50 mg/kg)* (◇), *aspirin* (○) *and acetaminophen* (▲) *(250 and 125 mg/kg, respectively), and aspirin* (●) *and caffeine* (♦) *(250 and 50 mg/kg, respectively). Values are means* ± SEM *from eight rats. An asterisk indicates* $p < 0.05$ *compared to the drugs given alone.*

RESULTS

GLC—Linear relationships were observed between the concentrations of aspirin or salicylic acid (5–100 nmoles/ml) added to normal rat plasma and the peak area ratio measured using *p*-toluic acid as the internal standard ($r = 0.9989$ for aspirin and 0.9976 for salicylic acid). The mean recoveries of added aspirin and salicylic acid from plasma were 98.6 ± 3.5 and 99.9 ± 5.8%, respectively. The recoveries from tissue homogenates were 98.2 ± 3.4 and 99.5 ± 5.0%, respectively ($n = 8$). The detection limit for both compounds was 2 nmoles/ml of plasma; acetaminophen, caffeine, and phenacetin did not interfere with the assay.

In the concentration range of 4–100 nmoles/ml, linear relationships were observed between the concentrations of acetaminophen or phenacetin added to blood plasma and the peak area ratio measured using *p*-bromoacetanilide as the internal standard ($r = 0.9998$ for acetaminophen and 0.9993 for phenacetin). The mean recoveries of acetaminophen and phenacetin from plasma were 70.0 ± 4.0 and 99.0 ± 5.0%, respectively. The recoveries from tissue homogenates were 71.2 ± 4.2 and 99.3 ± 5.3%, respectively ($n = 15$). The detection limit for both compounds was 0.5 nmole/ml of plasma; aspirin, salicylic acid, caffeine, and *p*-aminophenol did not interfere with the assay.

A linear relationship was observed between the concentrations of caffeine (5–50 nmoles/ml) added to blood plasma and the peak area ratio measured using phenacetin as the internal standard ($r = 0.9974$). The recovery of caffeine from plasma samples in the concentration range of 1–175 nmoles/ml was 95.0 ± 4.4% ($n = 17$). The recovery from tissue homogenates in the concentration range of 20–500 nmoles/g was 94.1 ± 5.0% ($n = 8$). The detection limit was 1 nmole/ml of plasma; aspirin, salicylic acid, and acetaminophen did not interfere with the assay.

Plasma Levels during Elimination—The average plasma levels of salicylate, acetaminophen, and caffeine after administration to rats of the individual drugs alone or in combination are shown in Fig. 1.

Administration of aspirin alone or in combination with acetaminophen

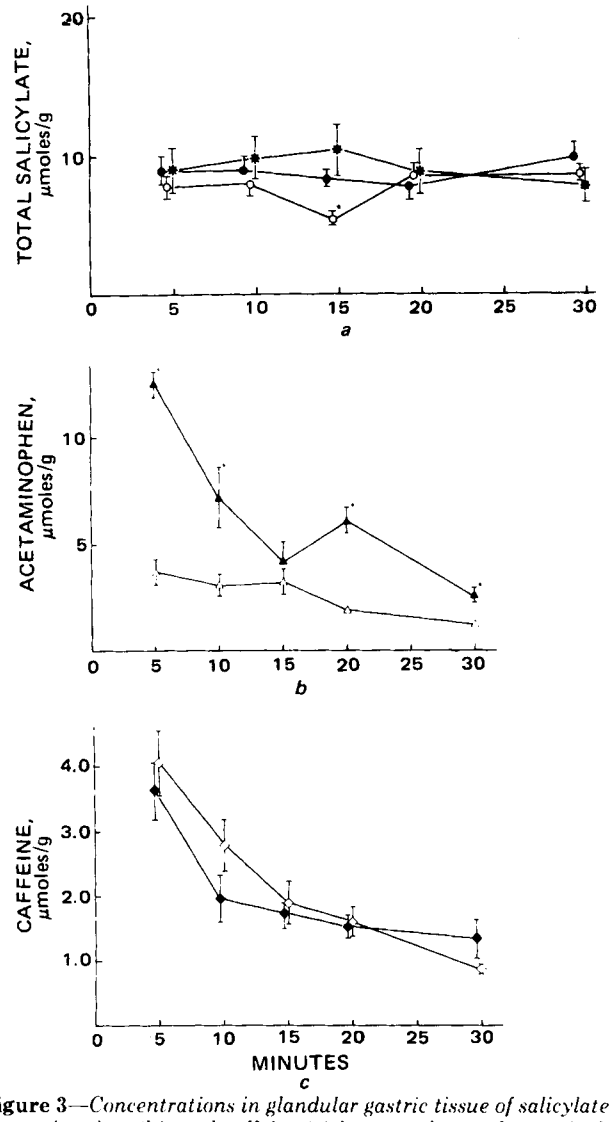

Figure 3—*Concentrations in glandular gastric tissue of salicylate* (a), *acetaminophen* (b), *and caffeine* (c) *in starved rats after oral administration of aspirin (250 mg/kg)* (*), *acetaminophen (125 mg/kg)* (△), *caffeine (50 mg/kg)* (◇), *aspirin* (○) *and acetaminophen* (▲) *(250 and 125 mg/kg, respectively), and aspirin* (●) *and caffeine* (◆) *(250 and 50 mg/kg, respectively). Values are means* ± SEM *from eight rats. An asterisk indicates* $p < 0.05$ *compared to the drugs given alone.*

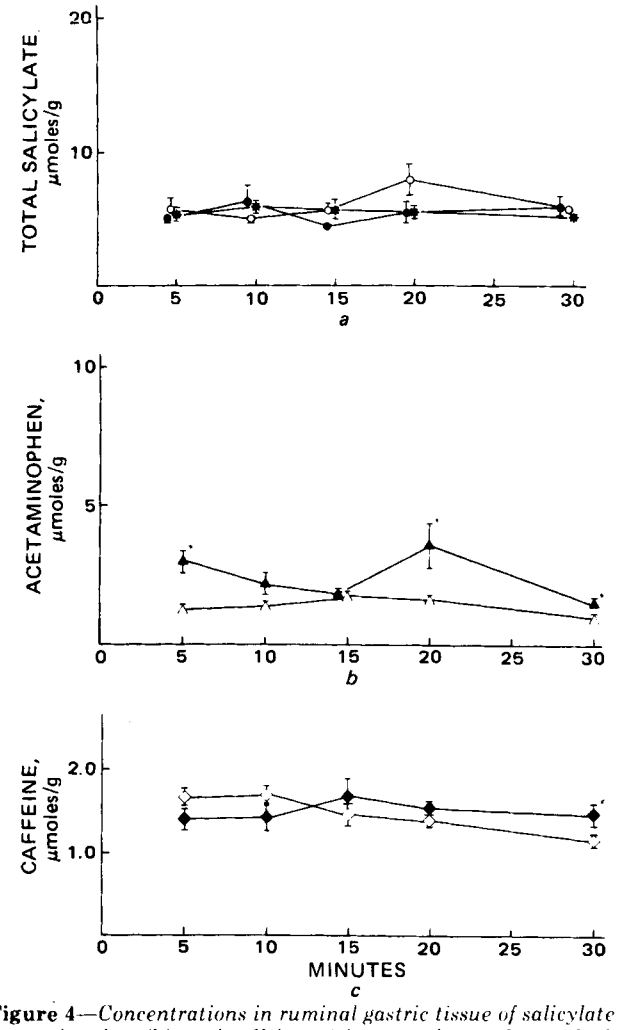

Figure 4—*Concentrations in ruminal gastric tissue of salicylate* (a), *acetaminophen* (b), *and caffeine* (c) *in starved rats after oral administration of aspirin (250 mg/kg)* (*), *acetaminophen (125 mg/kg)* (△), *caffeine (50 mg/kg)* (◇), *aspirin* (○) *and acetaminophen* (▲) *(250 and 125 mg/kg, respectively), and aspirin* (●) *and caffeine* (◆) *(250 and 50 mg/kg, respectively). Values are means* ± SEM *from eight rats. An asterisk indicates* $p < 0.05$ *compared to the drugs given alone.*

or caffeine resulted in maximal plasma salicylate levels within 1.0–1.5 hr. Coadministration of acetaminophen with aspirin significantly reduced the plasma salicylate levels at 0.5 hr, whereas the levels after 1.5 hr were enhanced significantly. Simultaneous administration of caffeine with aspirin significantly reduced plasma salicylate levels at 1.5 hr after administration (Fig. 1*a*).

In the presence of aspirin, the plasma levels of acetaminophen (Fig. 1*b*) and caffeine (Fig. 1*c*) were decreased significantly in comparison to the levels following administration of the individual drugs.

Plasma and Gastric Tissue Concentrations during Absorption—*Plasma Levels*—The average plasma levels of salicylate, acetaminophen, and caffeine during their absorption are shown in Fig. 2 after administration of the individual drugs alone or in combination to rats.

In comparison with aspirin treatment alone, administration of mixtures of aspirin and acetaminophen and aspirin and caffeine resulted in significantly lower plasma salicylate levels during the first few minutes of absorption. However, at $t = 30$ min, the salicylate levels in the presence of caffeine equaled those after aspirin treatment alone; in the presence of acetaminophen, the plasma salicylate levels were enhanced at $t = 20$ and 30 min (Fig. 2*a*). With aspirin, the plasma levels of acetaminophen (Fig. 2*b*) and caffeine (Fig. 2*c*) were reduced significantly during absorption.

Concentrations in Glandular Gastric Tissue—The average concentrations of salicylate, acetaminophen, and caffeine in the glandular tissue of the rat stomach during absorption are presented in Fig. 3 after administration of the individual drugs alone or in combination.

Maximal concentrations of salicylate in the glandular gastric tissue were observed within 5–15 min after administration of aspirin (Fig. 3*a*). Simultaneous administration of acetaminophen with the aspirin tended to retard salicylate absorption, although statistically significant differences between the glandular tissue concentrations could be demonstrated only at $t = 15$ min. Coadministration of caffeine with aspirin did not affect the absorption of salicylate into the glandular tissue.

Acetaminophen (Fig. 3*b*) and caffeine (Fig. 3*c*) were rapidly absorbed into the glandular part of the rat stomach. In the presence of aspirin, the absorption of acetaminophen into the glandular tissue increased significantly whereas the absorption of caffeine was not affected.

During the absorption phase, the ratios of the glandular tissue concentration to the plasma concentration (Table I) remained almost constant for salicylate; for acetaminophen and caffeine, this ratio gradually decreased with time. Coadministration of acetaminophen did not influence the glandular tissue to plasma salicylate concentration ratio; conversely, this ratio for the acetaminophen concentration significantly increased in the presence of aspirin (Table I). Simultaneous administration of caffeine with aspirin tended to increase the concentration ratios for both drugs, although significant differences were observed only at $t = 15$ min for salicylate and at $t = 30$ min for caffeine.

Concentrations in Ruminal Gastric Tissue—The average concentrations of salicylate, acetaminophen, and caffeine in the ruminal tissue of the rat stomach during absorption are presented in Fig. 4 after administration of the individual drugs alone or in combination.

Table I—Mean Concentration Ratios (w/v) of Glandular Gastric Tissue to Blood Plasma following Oral Administration of Drugs and Drug Mixtures to Starved Rats (n = 8)

Treatment	Substance Determined	Time after Administration, min				
		5	10	15	20	30
Aspirin, 250 mg/kg	Salicylate	6.0	6.5	4.9	5.7	6.3
Acetaminophen, 125 mg/kg	Acetaminophen	10.1	8.6	8.1	5.6	3.3
Caffeine, 50 mg/kg	Caffeine	29.0	14.9	8.1	7.9	4.4
Aspirin and acetaminophen, 250 and 125 mg/kg	Salicylate	6.8	7.5	4.2	4.9	4.7
	Acetaminophen	45.5[a]	34.2[a]	18.7[a]	27.4[a]	14.9[a]
Aspirin and caffeine, 250 and 50 mg/kg	Salicylate	12.4	6.8	7.8[b]	6.1	6.5
	Caffeine	40.4	15.6	11.9	10.9	9.6[c]

[a] $p < 0.05$ compared to acetaminophen alone. [b] $p < 0.05$ compared to aspirin alone. [c] $p < 0.05$ compared to caffeine alone.

Table II—Mean Concentration Ratios (w/v) of Ruminal Gastric Tissue to Blood Plasma following Oral Administration of Drugs and Drug Mixtures to Starved Rats (n = 8)

Treatment	Substance Determined	Time after Administration, min				
		5	10	15	20	30
Aspirin, 250 mg/kg	Salicylate	4.6	3.7	3.3	3.5	4.1
Acetaminophen, 125 mg/kg	Acetaminophen	3.0	4.1	4.4	5.3	2.8
Caffeine, 50 mg/kg	Caffeine	12.0	9.0	6.2	6.9	5.9
Aspirin and acetaminophen, 250 and 125 mg/kg	Salicylate	4.9	4.6	4.2	4.5	3.3
	Acetaminophen	11.2[a]	10.5[a]	7.9[a]	15.9[a]	8.9[a]
Aspirin and caffeine, 250 and 50 mg/kg	Salicylate	7.0	4.5	4.2	4.4	4.1
	Caffeine	15.4	11.1	11.6[b]	11.0[b]	11.1[b]

[a] $p < 0.05$ compared to acetaminophen alone. [b] $p < 0.05$ compared to caffeine alone.

Table III—Mean Concentration Ratios (w/w) of Glandular Tissue to Ruminal Tissue of the Stomach following Oral Administration of Drugs and Drug Mixtures to Starved Rats (n = 8)

Treatment	Substance Determined	Time after Administration, min				
		5	10	15	20	30
Aspirin, 250 mg/kg	Salicylate	1.6	1.5	1.6	1.6	1.6
Acetaminophen, 125 mg/kg	Acetaminophen	3.5	2.5	2.2	1.4	1.6
Caffeine, 50 mg/kg	Caffeine	2.4	1.6	1.3	1.1	0.8
Aspirin and acetaminophen, 250 and 125 mg/kg	Salicylate	1.7	1.6	1.0	1.4	1.5
	Acetaminophen	4.9	3.4	2.3	2.1	1.7
Aspirin and caffeine, 250 and 50 mg/kg	Salicylate	1.8	1.9	1.9	1.6	1.8
	Caffeine	2.6	1.4	1.1	1.0	0.9

Aspirin administration, alone as well as in combination with acetaminophen or caffeine, resulted in comparable ruminal salicylate concentrations, which remained constant during the absorption phase (Fig. 4*a*). As in the glandular tissue, the absorption of acetaminophen into the rumen (Fig. 4*b*) was increased significantly in the presence of aspirin whereas the absorption of caffeine (Fig. 4*c*) was not affected.

The ratio of the ruminal tissue concentration to the plasma concentration (Table II) remained almost constant for salicylate and acetaminophen throughout the absorption phase; for caffeine, this ratio gradually decreased with time. The concentration ratios for salicylate were not affected by coadministration of acetaminophen or caffeine; on the other hand, aspirin enhanced the concentration ratios for both acetaminophen and caffeine (Table II).

Glandular Tissue to Ruminal Tissue Concentration Ratios—Table III shows the average glandular tissue to ruminal tissue concentration ratios for salicylate, acetaminophen, and caffeine during their absorption following administration of the individual drugs alone or in combination.

Regardless of whether the drugs were administered separately or in combination, the absorption of all drugs into the glandular tissue was higher than into the rumen. In particular, acetaminophen showed marked accumulation in the glandular tissue. Administration of the drug combinations did not affect the accumulation of the individual drugs in the glandular tissue.

DISCUSSION

Analytical Procedure—The assay used for aspirin and salicylic acid was a modification of earlier methods (12–16). By making derivatives of aspirin and salicylic acid with pure bis(trimethylsilyl)trifluoroacetamide under controlled conditions, more reproducible results were obtained in comparison with methods where the derivatizing agent was diluted with carbon disulfide (12) or acetone (15). With the temperature programming used, an acceptable separation of the desired compounds was obtained from endogenous compounds in plasma and gastric tissue homogenates. Care had to be taken during evaporation of the organic solvent to prevent sublimation (76°) of the aspirin and salicylic acid.

Many stationary phases, such as FFAP (17), OV-1 (18), SP 2250 (19), and OV-17 (20), can be used for the GLC determination of acetaminophen. Because an assay was needed that also could be used in investigations with phenacetin, one assay for both compounds was developed; the use of *p*-bromoacetanilide as an internal standard (21, 22) and an OV-101 column gave the best quantitative results.

Several previous investigations (23–25) involved the determination of caffeine in plasma; none of these reports described a method suitable for tissues. In the present assay, an OV-101 column (24) was used because with an OV-17 column (25) and hexobarbital and phenacetin as internal standards, both compounds gave broad tailing peaks. Extracting caffeine with dichloromethane instead of chloroform (24, 25) rendered the procedure more convenient and less hazardous.

Glandular Accumulation—One theory explaining the gastric toxicity of aspirin assumes an association between its erosive activity and its toxic intracellular actions after absorption (3, 26). Although orally administered aspirin is absorbed mainly from the small intestine (27), considerable amounts also are absorbed *via* the stomach (28). Under the acidic conditions present in the stomach, aspirin (pKa 3.5) is mainly undissociated, which facilitates diffusion of the drug molecules across membranes. In mucosal cells, aspirin is likely to be hydrolyzed to salicylic acid (29), and this compound exists predominantly in an ionized form in the cells (pH ~7.4). Because the back-diffusion of salicylate ions across membranes is very slow, high intracellular concentrations of salicylate rapidly accumulate (30, 31). If sufficiently high concentrations are reached, mitochondrial oxidative phosphorylation is uncoupled, resulting in cessation of synthetic and transport activities in the cell and possibly leading to a reduction in transmucosal resistance (32). However, not all cell types of the gastric mucosa appear to be equally susceptible to damage since erosions induced with aspirin are found only in the glandular part of the rat stomach. Recently, Brune *et al.* (5) showed that the

parietal cells in the glandular mucosa trap salicylate to an excessive extent. It is possible that gastric mucosal damage induced with aspirin starts with the decay of these cells.

In the present study, the salicylate concentrations reached in the glandular tissue during aspirin absorption were higher than those in the rumen (Table III). The concentrations reached in the gastric mucosa depend on the presence of the drug within the stomach lumen since lower levels are obtained in the mucosa after subcutaneous administration of aspirin (33). Acetaminophen (pKa 9.5) and caffeine (pKa 13.2) also accumulated in the glandular tissue after oral administration, to an even larger extent than salicylate (Table III). The fact that acetaminophen and caffeine do not induce gastric erosions (6) might indicate that the intracellular action of these compounds differs from that of salicylate. Thus, accumulation of a drug in glandular tissue *per se* does not account for erosive activity. However, since the erosions are found only in the glandular part of the stomach, in which the highest concentrations of salicylate were reached, an involvement of salicylate accumulation in glandular tissue in the pathogenesis of aspirin-induced erosions cannot be ruled out.

Throughout the absorption period after aspirin treatment, salicylate concentrations in the glandular and ruminal areas of the stomach were not significantly affected by simultaneous administration of either acetaminophen or caffeine (Figs. 3*a* and 4*a*). Only in the group of rats killed after 15 min did salicylate levels in the glandular tissue tend to decrease in the presence of acetaminophen. However, in view of the large number of different observations made at various time points, the possibility that one mean is (significantly) lower by chance is not inconceivable. In any case, the concentrations of salicylate still achieved values of 6–10 μmoles/g in the presence of acetaminophen. Exposure of the gastric mucosa to similar concentrations of salicylate, both *in vitro* (34, 35) and *in vivo* (36, 37), is associated with changes in its electrophysiological properties and permeability characteristics. Furthermore, oral administration of aspirin (50 mg/kg), leading to salicylate concentrations in gastric tissue of 1–2 μmoles/g (33), still causes gastric erosions (6). Hence, it is unlikely that this slight reduction in salicylate accumulation in glandular tissue accounts for the inhibition of the erosive activity of aspirin by acetaminophen.

In the presence of aspirin, the absorption of acetaminophen into the gastric tissues increased (Figs. 3*b* and 4*b*), and this increased absorption might have affected prostaglandin biosynthesis in the gastric wall. According to Vane (38), inhibition of prostaglandin biosynthesis in the gastric mucosa by aspirin-like drugs at least contributes to the formation of gastric erosions. In addition, coadministration of prostaglandins protects the gastric mucosa against irritation by aspirin (39). Therefore, the observation of Robak *et al.* (40) that acetaminophen, in concentrations similar to those measured in blood plasma, stimulates prostaglandin synthetase activity *in vitro* might be important. As a result of its (increased) accumulation, acetaminophen might protect against the erosive activity of aspirin by counteracting the aspirin-induced inhibition of prostaglandin biosynthesis in the glandular part of the stomach.

Blood Plasma Concentrations—The combination of acetaminophen with aspirin retarded the appearance of salicylate in plasma (Fig. 2*a*), and the plasma salicylate levels were enhanced during elimination. A possible explanation for the latter observation might be competition of aspirin with acetaminophen for the glucuronide-forming enzyme system. Such a metabolic interaction between salicylate and acetaminophen could not be demonstrated in humans (41) or rats (42). However, in these studies, considerably lower doses of both drugs were used compared to those used in the present study. In both previous reports (41, 42), the investigators indicated that such an interaction at higher doses may be possible.

The combination of aspirin with acetaminophen appeared to reduce the acetaminophen absorption rate (Fig. 2*b*), an observation that has been reported for the rat (43), guinea pig (44), hamster (45), and mouse (46). Presumably, this retardation is related to the rate of gastric emptying. In this context, Smith and Irving (47) reported that salicylate administered to rats reduced the gastric emptying rate, and it was demonstrated recently that reduction of the gastric emptying rate reduced acetaminophen absorption (48–50). The increment in the acetaminophen concentrations in the glandular and ruminal areas of the stomach corroborates this assumption. After coadministration with aspirin, the elimination rate of acetaminophen from plasma was lower than that of acetaminophen given alone (Fig. 1*b*). This phenomenon might be due to retardation of the acetaminophen absorption and/or to an aspirin-induced decrease in the elimination rate of acetaminophen (43).

Administration of aspirin with caffeine retarded the appearance of both drugs in plasma (Figs. 2*a* and 2*c*). This effect probably is due to a delay in gastric emptying since both caffeine and aspirin relax GI smooth muscles (51, 52). Since simultaneous administration of aspirin and caffeine did not affect the concentrations of either of these drugs in the gastric wall (Figs. 3*a*, 3*c*, 4*a*, and 4*c*), the appearance of salicylate and caffeine in plasma probably depends mainly on absorption from the small intestine and not on absorption from the stomach.

In summary, neither the inhibition by acetaminophen nor the potentiation by caffeine of aspirin-induced erosions in the rat stomach can be attributed to effects of these drugs on salicylate accumulation in glandular mucosa. Furthermore, since simultaneous administration of these drugs to the rat influenced their mutual rates of absorption and elimination, the pharmacological efficacy of the mixtures cannot be predicted simply from the plasma levels of the components.

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Rearrangement of Chloramphenicol-3-monosuccinate

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Abstract □ The equilibrium mixture of chloramphenicol-3-monosuccinate and its alternate form at neutral pH in aqueous solution was reexamined. The structure of the alternate form was shown by mass spectrometry and NMR spectroscopy to be chloramphenicol-1-monosuccinate and not the cyclic hemi-ortho ester reported previously.

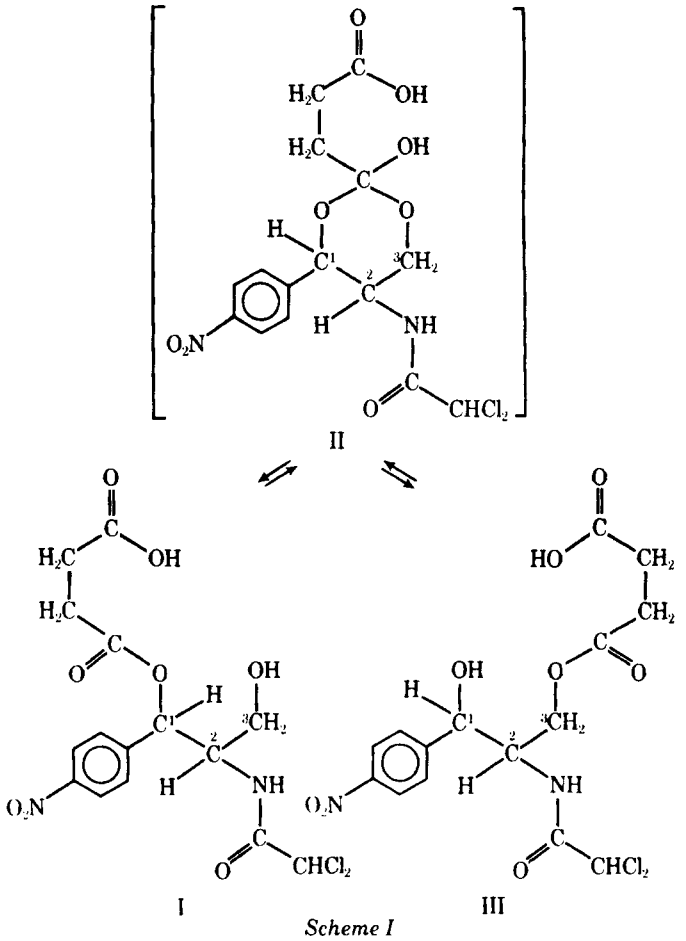
Keyphrases □ Chloramphenicol-3-monosuccinate—identification of alternate form in equilibrium mixture as chloramphenicol-1-monosuccinate □ Antibacterials—chloramphenicol, identification of 1-succinate ester as alternate form in equilibrium mixture with 3-succinate ester □ Prodrugs—chloramphenicol-3-monosuccinate, identification of 1-succinate ester as alternate form in equilibrium mixture with 3-succinate ester

The sodium salt of chloramphenicol-3-monosuccinate[1] (III) is used as a prodrug, generating the broad spectrum antibiotic chloramphenicol by hydrolysis of the succinate ester in the liver (1). Sandmann *et al.* (2) reported that at neutral pH, III exists in equilibrium with a different molecular form, which they identified as a cyclic hemi-ortho ester (II) (Scheme I).

RESULTS AND DISCUSSION

By using a recently developed, sensitive, high-pressure liquid chromatographic (HPLC) assay for III, three products were detected after equilibrating III in aqueous solution at pH 7.5 for 24 hr (3). The first and third peaks (Fig. 1) were identified as chloramphenicol and III, respectively, by comparison with standards and by mass spectrometry of their trimethylsilyl ethers. The mass spectrum of the trimethylsilyl derivative of the first HPLC peak agreed with spectra published for *O*-bis(trimethylsilyl)chloramphenicol (4–6). The mass spectrum of the trimethylsilyl derivative of the third HPLC peak gave ions consistent with IV: *m/e* 551 (2.6%, M − CH_3), 483 (0.6), 377 (2.92), 225 (75.6), 224 (28.9), 173 (25.2), and 73 (100.0).

The second eluted compound exhibited an NMR spectrum identical to that reported by Sandmann *et al.* (2) for II. However, the mass spectrum of the trimethylsilyl derivative of this compound supported the structure of chloramphenicol-1-monosuccinate (I) and not the cyclic hemi-ortho ester (II). The ion having the greatest mass was *m/e* 551 (1.7%,



Scheme I

[1] The USAN name chloramphenicol sodium succinate refers to the sodium salt of the 3-monosuccinate ester.

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