Effect of Concurrent Administration of Choline Salicylate and Acetaminophen on Their Mutual Biotransformation in the Rat

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Abstract □ Rats were administered either choline salicylate (2 mg./kg.) or acetaminophen (10 mg./kg.) or a combination of both by gastric intubation. Plasma levels of salicylic acid and acetaminophen were measured at various time intervals by suitable modifications of reported analytical methods. Concurrent administration of the two drugs did not alter the plasma salicylic acid levels but resulted in a slight increase of the plasma acetaminophen values at 5 and 10 min. Differing degrees of glucuronidation of the two drugs may explain this interaction which, however, appears too low to be of clinical significance.

Keyphrases □ Choline salicylate and acetaminophen, concurrent administration—effect on individual plasma levels, rats □ Acetaminophen and choline salicylate, concurrent administration—effect on individual plasma levels, rats □ Analgesics, concurrent administration of choline salicylate and acetaminophen—effect on individual plasma levels, rats □ Plasma levels—choline salicylate and acetaminophen after concurrent administration, rats

Mutual inhibition of the metabolism of drugs sharing a common biotransformation pathway is well documented (1–3). Among the nonprescription analgesics, such interactions have been reported to occur between salicylamide and acetaminophen (4) and salicylic acid and salicylamide (5). In man, salicylic acid is metabolized to salicyluric acid, salicyl acylglucuronide, phenolic glucuronide, and gentisic acid (6). The main metabolic pathway of acetaminophen is conjugation to the glucuronide and sulfate (7). It is conceivable that salicylic acid and acetaminophen would compete for sites on the enzyme systems responsible for their biotransformation, this competition leading to decreased glucuronide formation which, in turn, might result in increased blood levels of the free drug(s). Levy and Regårdh (8) observed little interaction in the biotransformation of acetaminophen and salicylic acid in human volunteers. In their study, acetaminophen and sodium salicylate were given at different time intervals, and the urinary excretion rates of the metabolites were studied. Amsel and Davison (9) administered acetaminophen with single and multiple doses of aspirin and observed no effect on acetaminophen metabolism in man.

The present study was initiated to determine if the plasma levels of acetaminophen and salicylic acid are affected by concurrently administering the former with choline salicylate to rats. This experimental design was chosen because: (*a*) choline salicylate has been reported to be absorbed much faster, giving rise to higher blood levels of salicylic acid than an equivalent dose of aspirin in man (10, 11); and (*b*) in experimental animals like the rat, it is more convenient and amenable to measure quantitatively plasma drug levels at various intervals like 5, 10, and 15 min. than urinary excretion rates.

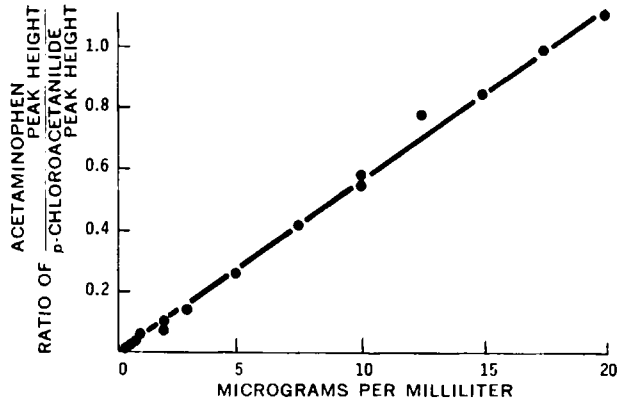

Figure 1—*Calibration curve of acetaminophen added to rat plasma.*

EXPERIMENTAL

Materials—The following were used: dichloroethane, spectroquality[1]; ethyl acetate, spectroquality[1]; phosphate buffers, 1.0 *M*, pH 8.0, and 0.1 *M*, pH 7.0; sodium sulfate, anhydrous, reagent grade[2]; *p*-chloroacetanilide[3]; nitrogen gas, high purity[4]; bis(trimethylsilyl)trifluoroacetamide[5]; pyridine[6]; choline salicylate, 51% (w/w) aqueous solution[6]; a column conditioner containing trimethylsilyl donors[7]; phenyl methyl silicone, 50% phenyl (OV-17)[8]; and Gas Chrom Q[8].

Methods—*Determination of Acetaminophen in Rat Plasma*—The method of Prescott (12) was modified and adopted as follows. Rat plasma (2.0 ml.) was adjusted to pH 8.0 by the addition of 1.0 ml. phosphate buffer, 1.0 *M*, pH 8.0, and then extracted by shaking for 10 min. with 6.0 ml. ethyl acetate. The mixture was centrifuged for 5 min. at 6000 r.p.m. The organic layer was separated and dried over 1.0 g. anhydrous sodium sulfate. A 4.0-ml. aliquot of the extract was transferred to a screw-capped conical tube containing 15 mcg. *p*-chloroacetanilide in ethyl acetate. The solvent was evaporated by heating at 60° under a stream of dry nitrogen. The sides of the tube were washed with 1.0 ml. ethyl acetate, and the solvent was again removed by evaporation. The residue was dissolved in 15 μl. pyridine, reacted with 15 μl. bis(trimethylsilyl)trifluoroacetamide at 60° for 30 min., and cooled; then 1.0 μl. was injected into a gas chromatograph[9] equipped with a flame-ionization detector.

The chromatographic conditions were: glass column, 0.63 cm. (0.25 in.) o.d., 1.82 m. (6 ft.), containing 10% phenyl methyl silicone (50% phenyl) on Gas Chrom Q (100–120 mesh); carrier gas (nitrogen) at a flow rate of 45 ml./min.; column temperature, 160°; injection block temperature, 210°; and detector temperature, 220°. The retention times of *p*-chloroacetanilide and acetaminophen were 4.0 and 7.8 min., respectively.

Determination of Salicylic Acid in Rat Plasma—The spectrofluorometric method of Rowland and Riegelman (13) was modified as follows. Plasma (2.0 ml.) was acidified to pH 2.0 with 3.0 *N* HCl and extracted with dichloroethane (25 ml.). Salicylic acid from

[1] Matheson, Coleman and Bell, Norwood, Ohio.
[2] J. T. Baker Co., Phillipsburg, N. J.
[3] Eastman Organic Chemicals, Rochester, N. Y.
[4] SOS Gases Inc., Kearny, N. J.
[5] Regisil, Regis Chemical Co., Chicago, Ill.
[6] RSA Corp., Ardsley, N. Y.
[7] Silyl-8, Pierce Chemical Co., Rockford, Ill.
[8] Applied Science Laboratories Inc., State College, Pa.
[9] Perkin-Elmer model 900.

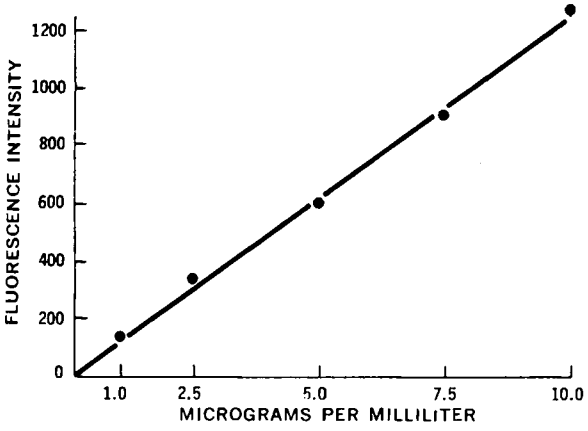

Figure 2—*Calibration curve of salicylic acid added to rat plasma.*

a 20-ml. aliquot of the organic layer was reextracted into 5.0 ml. of phosphate buffer, 0.1 *M*, pH 7.0, and the fluorescence was measured in a spectrophotofluorometer[10]. Activation and emission wavelengths were 302 and 406 nm., respectively, and both slit dials were set at 4.0 nm.

Separation and Estimation of Acetaminophen and Salicylic Acid from Rat Plasma in the Presence of Each Other—By employing the two procedures already outlined, a method was developed for the separation and estimation of acetaminophen and salicylic acid in plasma.

Two milliliters of plasma was extracted by shaking with ethyl acetate and analyzed for acetaminophen by the GLC procedure already described. The aqueous layer (2.5 ml.) was then reextracted with 6.0 ml. of ethyl acetate to remove residual acetaminophen in solution. The organic layer was discarded, and 2.0 ml. of the aqueous phase was acidified with 0.2 ml. of 6 *N* HCl and extracted with 25 ml. of dichloroethane. The salicylic acid was taken into phosphate buffer, 0.1 *M*, pH 7.0, and the fluorescence was measured as described earlier.

Plasma Levels of Acetaminophen and Salicylic Acid in Rats—Groups of 10 female Sprague–Dawley rats, 220–260 g., were fasted for 18 hr. They then received, by stomach tube, aqueous solutions of: (*a*) acetaminophen, 10 mg./kg.; (*b*) choline salicylate, 2 mg./kg.; or (*c*) a combination of acetaminophen, 10 mg./kg., and choline salicylate, 2 mg./kg. Blood samples were withdrawn into heparinized tubes by cardiac puncture immediately prior to and at 5, 10, 15, 30, 45, 60, 90, and 120 min. after treatment. Ten rats were used per time period. The plasma was separated by centrifugation and analyzed for acetaminophen and/or salicylic acid according to one of the three described procedures.

RESULTS

Determination of Acetaminophen in Rat Plasma—A linear relationship was observed between the concentration of acetaminophen (0.05–20 mcg./ml.) and the peak height ratio (peak height of acetaminophen/peak height of *p*-chloroacetanilide) when known amounts were added to normal rat plasma and assayed by the analytical procedure (Fig. 1). The recovery of added drug was 67%. It was found that the column efficiency could be maintained better by periodic injections of five 10-μl. portions of column conditioner[7] at 60° and programming the column to 200° at 2°/min. with its detector end disconnected after every 20–30 analyses.

Known amounts of salicylic acid (1.0–10 mcg./ml.) were added to rat plasma and assayed. A linear relationship was observed between the salicylic acid concentration and the fluorescence (Fig. 2). The recovery was 100%.

Varying concentrations of acetaminophen (1–20 mcg./ml.) were added to rat plasma in the presence of 20 mcg. of salicylic acid, as were known concentrations of salicylic acid (0.5–20 mcg./ml.) in the presence of 20 mcg. of acetaminophen. Then the samples were assayed differentially according to the procedure already outlined. There was no interference by either drug on the recovery or measurement of the other. Consequently, the calibration curves shown in Figs. 1 and 2 were employed to determine the concentrations of the two drugs in plasma samples containing the two in unknown proportions.

[10] Perkin-Elmer model MPF-3.

Table I—Plasma Levels[a] of Salicylic Acid (Micrograms per Milliliter) after Oral Administration of Choline Salicylate Either Alone or in Combination with Acetaminophen

Time Postdrug, min.	Choline Salicylate, 2.0 mg./kg.	Choline Salicylate, 2.0 mg./kg., plus Acetaminophen, 10 mg./kg.
0	0	0
5	6.9 ± 0.6	6.7 ± 0.6
10	6.1 ± 0.3	6.7 ± 0.4
15	6.6 ± 0.2	6.8 ± 0.6
30	5.8 ± 0.3	5.5 ± 0.4
45	5.3 ± 0.3	5.9 ± 0.4
60	4.9 ± 0.5	5.4 ± 0.4
90	4.6 ± 0.2	4.9 ± 0.5
120	4.4 ± 0.3	4.6 ± 0.5

[a] Average of 10 individual experiments ± *SE*.

Plasma Levels of Acetaminophen and Salicylic Acid in Rats—The average plasma levels of salicylic acid and acetaminophen attained after administration of either drug alone or in combination to rats are shown in Tables I and II, respectively.

Oral administration of choline salicylate (2 mg./kg.) to rats resulted in peak salicylic acid plasma levels (6.9 mcg./ml.) within 5 min., which slowly decreased over 2 hr. to 4.4 mcg./ml. at 120 min. Concurrent administration with acetaminophen (10 mg./kg.) resulted in similar levels of salicylic acid, which were 6.7 mcg./ml. at 5 min., 6.8 mcg./ml. at 15 min., and 4.6 mcg./ml. at 120 min.

Acetaminophen (10 mg./kg.) administered orally to rats produced plasma levels that rose quickly to a peak (11.3 mcg./ml.) at 15 min. and then declined sharply to 0.1 mcg./ml. by 120 min. However, concurrent administration of acetaminophen (10 mg./kg.) with choline salicylate (2 mg./kg.) resulted in higher plasma acetaminophen levels at 5 and 10 min. ($0.05 < p < 0.1$ by a two-tail *t*-test).

DISCUSSION

Derivatizing acetaminophen by heating with bis(trimethylsilyl)-trifluoroacetamide under controlled conditions resulted in quantitative and more reproducible results. Employing dichloroethane in place of ether for extracting salicylic acid rendered the operation more facile and less hazardous. Therefore, the methodology appeared to be an improvement over existing ones (12, 13).

Levy and Regårdh (8) observed no interaction in man in the biotransformation of acetaminophen and salicylate on the basis of urinary excretion data. They did not rule out, however, the possibility of the inhibition of glucuronide and/or sulfate formation under forced or different conditions. Amsel and Davison (9) suggested that the two drugs might be metabolized by different systems in man.

The interaction between acetaminophen and salicylates in experimental animals has not been studied before. In the present study, concurrent administration of these two drugs to the rat resulted in slightly elevated plasma acetaminophen concentrations at 5 and 10 min. while the salicylate levels remained unaffected. The dose (per body weight) of acetaminophen given to rats compares favorably with that given to humans by earlier workers (8, 9). Salicylate was administered at a much lower dose because: (*a*) choline salicylate given orally resulted in peak plasma levels by 5 min. which slowly declined up to 120 min., and (*b*) too high plasma salicylate levels could so saturate the enzyme systems as to make it difficult to discern any alterations caused by acetaminophen. The interaction in rats, although it may be too low to be of any clinical significance, is possibly due to differing degrees of glucuronidation undergone by the two drugs in the rat. This is analogous to the inhibitory effect of benzoic acid on the formation of salicyluric acid from salicylate in man (14). Salicylic acid, on the other hand, was without an effect on hippuric acid formation from benzoic acid. The latter is almost completely biotransformed to hippuric acid, whereas only 50–70% of the former is conjugated with glycine in man. If it is assumed that a substrate or a drug with a greater affinity to the glucuronide-

Table II—Plasma Levels[a] of Acetaminophen (Micrograms per Milliliter) after Oral Administration of Acetaminophen Either Alone or in Combination with Choline Salicylate

Time Postdrug, min.	Acetaminophen, 10 mg./kg.	Acetaminophen, 10 mg./kg., plus Choline Salicylate, 2.0 mg./kg.
0	0	0
5	9.5 ± 0.9	11.4 ± 1.0[b]
10	9.0 ± 0.6	11.6 ± 1.3[b]
15	11.3 ± 2.8	8.5 ± 1.2
30	4.4 ± 0.3	4.5 ± 0.6
45	2.5 ± 0.3	3.5 ± 0.7
60	1.5 ± 0.2	1.5 ± 0.2
90	0.6 ± 0.1	0.6 ± 0.1
120	0.1 ± 0.03	0.3 ± 0.1

[a] Average of 10 individual experiments ± *SE*. [b] $0.05 < p < 0.1$ (by two-tail *t*-test).

forming enzyme system would be metabolized to a greater extent than one with a lower affinity, then salicylate appears to have a greater affinity and extent of glucuronide formation than acetaminophen in the rat.

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DRUG STANDARDS

Individual Tablet Analysis for Codeine and Caffeine in Codeine-Aspirin-Phenacetin-Caffeine Tablets

THERON JAMES

Abstract □ A procedure is reported for determining the codeine and caffeine content of individual codeine-aspirin-phenacetin-caffeine tablets. Codeine is determined fluorometrically after extraction into dilute sulfuric acid; caffeine is extracted from a chloroform solution of the remaining ingredients with phosphoric acid and determined by UV spectroscopy. Average recoveries with a synthetic mixture were 99.7 and 98.9% for codeine and caffeine, respectively. Assay results are reported for codeine [1–65 mg. (1/65–1 grain)/tablet] and caffeine [32 mg. (1/2 grain)/tablet] in several different commercial samples. The proposed procedure is also compared with official methods.

Keyphrases □ Codeine with aspirin-phenacetin-caffeine tablets—individual tablet analysis for codeine and caffeine, compared to official methods □ Aspirin-phenacetin-caffeine with codeine tablets—individual tablet analysis for codeine and caffeine, compared to official methods □ Spectrophotofluorometry—analysis, codeine in aspirin-phenacetin-caffeine with codeine tablets □ UV spectrophotometry—analysis, caffeine in aspirin-phenacetin-caffeine with codeine tablets □ Analgesic formulations—analysis of codeine and caffeine in individual aspirin-phenacetin-caffeine with codeine tablets

The NF XIII monograph (1) for tablets containing codeine and aspirin-phenacetin-caffeine (1) requires content uniformity tests for codeine and caffeine. The methods involve a GC determination for codeine, while caffeine is determined by UV spectroscopy after column chromatographic separation. Utilization of these procedures for content uniformity necessitates two separate analyses. Consequently, a single tablet is not tested for *both* drugs (*i.e.*, codeine is determined in one group of tablets while caffeine is determined in