Simultaneous Metabolism of Aspirin and Acetaminophen in Man

LEWIS P. AMSEL▲ and CLARKE DAVISON

Abstract □ Aspirin, administered orally in a single dose (2.9 g.) 1 hr. before 650 mg. acetaminophen, had no effect on the formation of the glucuronide and sulfate of acetaminophen. When aspirin was administered on a multiple-dose regimen of 2.6 g. daily for 3 days, followed by a 650-mg. dose of acetaminophen on the 4th day, there was likewise no effect on the formation of acetaminophen metabolites. Since large doses of aspirin have no apparent effect on acetaminophen metabolism, it appears these two drugs may be metabolized by different enzyme systems.

Keyphrases □ Aspirin, metabolism—effect of subsequent acetaminophen administration, enzyme systems involved, man □ Acetaminophen, metabolism—effect of prior aspirin administration, enzyme systems involved, man □ Analgesics—simultaneous metabolism of aspirin and acetaminophen, man

A number of previous reports have demonstrated mutual inhibition in the metabolism of nonnarcotic analgesics. These include acetaminophen and salicylamide (1), salicylic acid and salicylamide (2), and benzoate and salicylate (3). Levy and Regårdh (4) demonstrated a lack of interaction between two other analgesics: acetaminophen and salicylic acid. The lack of effect of salicylate upon acetaminophen metabolism has been suggested to be attributable to an insufficient dose of the former. Total doses of salicylic acid and acetaminophen used in the Levy and Regårdh study were 1.5 and 1.0 g., respectively. At approximately the same time, the present study was instituted, utilizing large single doses of aspirin as well as a multiple-dose schedule involving the administration of aspirin for 3 days. Since the most widely used analgesic is aspirin, this compound was studied in order for the results to be most applicable clinically. Also, since aspirin is frequently administered in a multiple-dose regimen, this aspect of possible interaction with acetaminophen was investigated. In addition to urine collections, blood samples were obtained from the subjects to elucidate further any pharmacokinetic interactions.

EXPERIMENTAL

The panels participating in these studies were made up of two and three healthy male subjects. In the single-dose study (two subjects), drugs were administered in the morning after an overnight fast. No food was permitted for 4 hr. In the control test, acetaminophen (650 mg.) was given as a flavored elixir with 120 ml. water. When aspirin was given with acetaminophen, it was administered as tablets in a dose of 2.9 g. 1 hr. before the dose of 650 mg. acetaminophen in solution. Urine was collected quantitatively at intervals for 12 hr. and assayed for acetaminophen as well as the glucuronide and sulfate conjugates.

The multiple-dose study consisted of administering aspirin as tablets in a dose of 2.6 g. daily for 3 days (two tablets four times daily). On the morning of the 4th day, an additional dose of 650 mg. aspirin as tablets was administered to the three subjects. One hour later, acetaminophen (650 mg.) in solution was ingested with 120 ml. water. Blood was collected during a 5-hr. period and assayed for acetaminophen.

Acetaminophen was determined in plasma by a GC procedure. Two milliliters of plasma was extracted by thorough stirring in a tube with 10 ml. of ethyl acetate containing 3.5 mcg./ml. of docosane as the internal standard. After centrifugation, the organic phase was removed as completely as possible and dried under a stream of air at 60°. A second extract with 10 ml. of plain ethyl acetate was made and pooled with the first. Standards were prepared in control plasma samples from 2.5 to 25 mcg./ml. The dried extracts were stirred with 0.1 ml. of silylating reagent consisting of 10 parts dry pyridine, 1 part hexamethyl disilazane, and 0.1 part trichloromethylsilane[1]. After 5–60 min., 3 μl. was injected on a GLC column, 1.8 m. × 0.3 cm. (6 ft. × 0.125 in.) i.d., packed with OV-17 on Gas Chrom Q. The oven temperature employed[2] was 190° with an inlet port temperature of 210° and a detector temperature of 215°. A flame-ionization detector was used with nitrogen flow. The retention time for acetaminophen was about 3.3 min.; for docosane, it was about 7 min. Calculations of the acetaminophen concentration was made from the ratio of the peak heights of acetaminophen to that of the docosane relative to the standard curve. Minimum sensitivity was about 1 mcg./ml. compound.

Urinary acetaminophen was determined by mixing 1 ml. urine with pH 5.5 buffer and extracting with ethyl acetate containing docosane. Samples were then treated and assayed using the plasma method. To the same buffered urine samples, a solution of Mylase-LH[3] (which is free of glucuronidase) was added, which will hydrolyze acetaminophen sulfate. The samples were allowed to remain at room temperature overnight and then extracted with ethyl acetate. They were then treated and assayed as already described. Standards

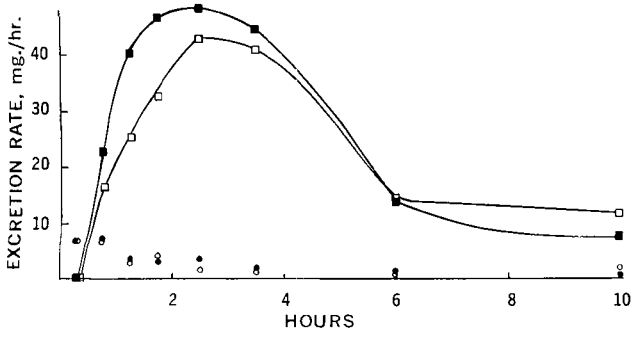

Figure 1—*Excretion rate as a function of time of acetaminophen glucuronide and unchanged acetaminophen in the presence and absence of aspirin. Key: acetaminophen glucuronide alone (■) and with aspirin (□), and unchanged acetaminophen alone (●) and with aspirin (○). Values are expressed as milligrams equivalent acetaminophen. (Mean data of Subjects L.A. and C.D.)*

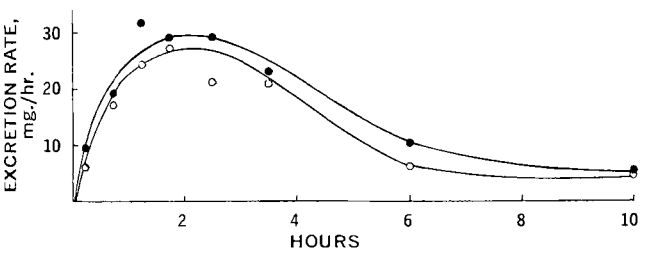

Figure 2—*Excretion rate as a function of time for acetaminophen sulfate alone (●) and with aspirin (○). Values expressed as milligrams equivalent acetaminophen. (Mean data of Subjects L.A. and C.D.)*

[1] Applied Sciences Laboratories, Inc.
[2] In the Packard model A30100.
[3] Wallerstein Laboratories, Morton Grove, Ill.

Table I—Recovery and Percent Excreted of Acetaminophen and Its Metabolites

Subject	Recovery, %		Expressed as Percent of Amount Recovered					
			Free Acetaminophen		Acetaminophen Sulfate		Acetaminophen Glucuronide	
	Control	With Aspirin	Control	With Aspirin	Control	With Aspirin	Control	With Aspirin
C.D.[a]	83.8	85.4	2.1	3.7	35.2	28.0	62.7	68.3
L.A.[a]	81.3	70.8	6.4	5.5	41.1	39.0	52.5	55.5
W.M.[b]	—[c]	85.7	—	5.6	—	41.9	—	52.4
M.D.[b]	—	106.3	—	5.3	—	40.9	—	53.9
E.C.[b]	—	78.8	—	4.1	—	33.2	—	62.7
Mean	82.6	85.4	4.3	4.8	38.2	36.6	57.6	58.6

[a] Dose of 650 mg. acetaminophen alone in one study and 1 hr. after a single dose of 2.9 g. aspirin in the other. [b] Dose of 2.6 g. aspirin daily for 3 days. On Day 4, 650 mg. acetaminophen was administered 1 hr. after 650 mg. of aspirin. [c] Urine not collected.

were made using authentic acetaminophen sulfate. β-Glucuronidase[4] was then added to the same urine sample and incubated at 37° for 4 hr. Samples were treated and assayed as already described to determine the amount of acetaminophen glucuronide. Standards for the latter assay were prepared by adding acetaminophen to control urine which had been preextracted for the free acetaminophen and sulfate assays.

Plasma salicylate and aspirin were determined by fluorescence after separation on a Sephadex G-10 column. One milliliter of plasma was precipitated with 3 ml. of tungstic acid solution (2 parts 10% $Na_2WO_4 \cdot 2H_2O$ plus 7 parts 0.19 *N* H_2SO_4). The tube was centrifuged and 2 ml. of the supernate was placed on a Sephadex column (12 × 0.9 cm. i.d.) which had been previously equilibrated with 0.02 *M* phosphate buffer, pH 6.6. The initial fluid was permitted to pass through, followed by 3 ml. of the buffer which was also discarded. Eight milliliters of buffer was next introduced and collected as the aspirin fraction. Twelve milliliters of distilled water was introduced and collected as the salicylate fractions. Both fractions were made basic with 0.5 ml. of 15 *N* NH_4OH and, after a few minutes, the fluorescence was measured on a spectrophotofluorometer[5]. An activation wavelength of 310 nm. was employed with fluorescent measurements at 400 nm. Standards ranging to 50 mcg./ml. of salicylate in control plasma were carried through the same procedure. Minimum sensitivity is about 0.5 mcg./ml.

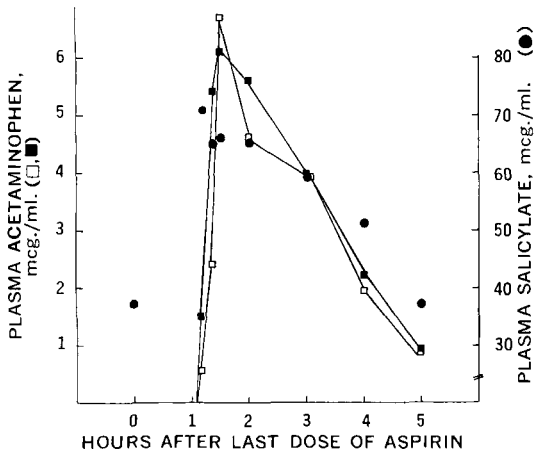

Figure 3—*Plasma levels of acetaminophen and salicylate as a function of time. Key: acetaminophen levels alone* (□) *and with aspirin* (■), *and plasma salicylate levels* (●) *obtained during the interaction study where aspirin was administered for 3 days with a final dose at 0 hr. on Day 4 (zero time in the figure). The dose of acetaminophen was administered 1 hr. after the last dose of aspirin. (Mean data of Subjects W.M., M.D., and E.C.)*

[4] Glusulase, Endo Laboratories, Garden City, N. Y.
[5] Aminco-Bowman.

RESULTS AND DISCUSSION

Figures 1 and 2 show the excretion rates of the two major metabolites of acetaminophen (the glucuronide and sulfate) both in the control study and when a single dose of aspirin was administered. Also shown in Fig. 1 is the excretion rate of unchanged acetaminophen for both studies. There was essentially no difference in the excretion patterns of the metabolic products in the presence or absence of aspirin. This confirms other work (4), where a similar lack of effect was noticed using a considerably lower dose of salicylate.

The 12-hr. recovery of urinary metabolites of acetaminophen is summarized in Table I. The percent of the dose recovered agrees well with previously reported data (4, 5), as does the low fraction of the dose excreted as unchanged acetaminophen. Table I also shows that there was essentially no difference in the percent excreted as the glucuronide and sulfate conjugates, as well as unchanged acetaminophen, when acetaminophen was administered alone or with aspirin. Thus the lack of effect of aspirin on acetaminophen metabolism is apparent even after high doses of aspirin.

To evaluate a more realistic situation, that is, one where a drug is taken continuously for a few days followed by another drug, the multiple-dose study was initiated. The multiple dosing with aspirin gave a fairly high level of salicylate in the plasma, as shown in Fig. 3. Also shown in the figure are the acetaminophen plasma levels both for the control study and when aspirin was given. There was apparently no effect of aspirin on acetaminophen plasma levels, even after continuous dosing with aspirin.

The urinary metabolites of acetaminophen after multiple dosing with aspirin are also shown in Table I. These are in general agreement with the control acetaminophen study, even though different subjects were utilized. These data also compare favorably with other work (4, 5). This lack of effect may be due to different metabolic systems which produce the metabolic end-products of acetaminophen and aspirin.

REFERENCES

(1) G. Levy and H. Yamada, *J. Pharm. Sci.*, **60,** 215(1971).
(2) G. Levy and J. A. Procknal, *ibid.*, **57,** 1330(1968).
(3) L. P. Amsel and G. Levy, *ibid.*, **58,** 827(1969).
(4) G. Levy and C. Regårdh, *ibid.*, **60,** 608(1971).
(5) A. J. Cummings, M. L. King, and B. K. Martin, *Brit. J. Pharmacol. Chemother.*, **29,** 150(1967).

ACKNOWLEDGMENTS AND ADDRESSES

Received January 10, 1972, from the *Sterling-Winthrop Research Institute, Rensselaer, NY 12144*

Accepted for publication April 14, 1972.

The authors thank Mrs. C. Coughlin for her excellent technical assistance.

▲ To whom inquiries should be directed.