tions. Thus, the desired reduction of k could be achieved at any temperature by adjusting the appropriate acetone vapor pressure. Conversely, at any vapor pressure the optimum release rate could be attained by manipulation of the treatment temperature. The extent of rate reduction would be limited only by condensation.

SUMMARY AND CONCLUSIONS

An apparatus was developed to study the variables involved in the acetone vapor treatment of tablets made from a methyl acrylate–methyl methacrylate copolymer matrix containing dispersed solid drug. This apparatus allows close control of temperature and acetone vapor pressure. It also allows monitoring of the amount of acetone absorbed throughout the run.

Release from the acetone-treated tablets followed the Higuchi relationship in which the quantity of drug released per unit surface area was proportional to the square root of time. Except under very mild conditions, the drug release rate was reduced. The extent of reduction was related to the amount of acetone absorbed. Generally, increasing the vapor pressure or decreasing the treatment temperature increased acetone absorption. Thus, by manipulation and control of these two variables, the drug release rate may be regulated in a reproducible fashion. The decrease in release rate can be explained primarily on the basis of an increase in matrix tortuosity.

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Drug Biotransformation Interactions in Man III: Acetaminophen and Salicylamide

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Abstract □ Acetaminophen (1 and 2 g.), salicylamide (1 g.), and 1 g. acetaminophen followed 1.5 hr. later by 1 or 2 g. salicylamide were administered in aqueous solutions to healthy human subjects. Urine was collected every 15–30 min. for the first 4–5 hr. and then at longer intervals. The urine samples were analyzed for acetaminophen, acetaminophen sulfate, acetaminophen glucuronide, salicylamide, salicylamide sulfate, and salicylamide glucuronide. The excretion rates of acetaminophen sulfate and acetaminophen glucuronide decreased immediately after administration of salicylamide. Acetaminophen decreased the formation of salicylamide sulfate. Coadministration of L-cysteine (a source of sulfate) prevented this effect and also increased the excretion of acetaminophen sulfate. The results indicate a competitive inhibition in the formation of acetaminophen sulfate and salicylamide sulfate and suggest also a competitive inhibition in the formation of acetaminophen glucuronide and salicylamide glucuronide.

Keyphrases □ Acetaminophen, salicylamide—biotransformation interaction, human □ Biotransformation interaction—acetaminophen, salicylamide □ Metabolites, acetaminophen, salicylamide—isolated, identified □ Colorimetric analysis—spectrophotometer

Previous reports from this laboratory have shown that the formation of salicylamide sulfate from salicylamide is easily saturated in man (1) and that there occurs a mutual inhibition of glucuronide formation by salicylamide and salicylate when these drugs are administered concomitantly (2). Since acetaminophen, like salicylamide, is eliminated primarily by glucuronide and sulfate formation, and since these mild analgesic and antipyretic drugs are often taken together, the effect of each on the biotransformation of the other has been investigated.

EXPERIMENTAL

Healthy, male, ambulatory human subjects received orally 1 or 2 g. acetaminophen in one test, 1 g. salicylamide in the second test, and 1 g. acetaminophen followed 1.5 hr. later by 1 or 2 g. salicylamide in the third test. Intervals between tests were at least 1 week. The drugs were administered in aqueous solution in the morning on an empty stomach. Food was withheld for at least 2 hr. thereafter. Urine was collected every 15–30 min. for the first 4–5 hr. and then at longer intervals for at least 24 hr. About 50 ml. of water was ingested after each urine collection to assure adequate urine output.

Determination of Acetaminophen and Its Metabolites in Urine—Free acetaminophen was extracted from urine by a method similar to that of Brodie and Axelrod (3). Acetaminophen glucuronide and acetaminophen sulfate were hydrolyzed enzymatically and then extracted as acetaminophen. The concentration of this drug was determined by a modification of the colorimetric method of Welch and Conney (4).

Specifically, free acetaminophen was extracted by adding 4 g. sodium chloride and 15 ml. ether[1] to 10 ml. urine. This mixture was shaken mechanically for 15 min. and centrifuged to separate the phases. Ten milliliters of the ether phase was extracted with 3 ml. 0.1 *N* sodium hydroxide solution by shaking 5 min. and centrifuging. One-half milliliter of the aqueous phase was pipeted into a graduated test tube, and 0.5 ml. 4 *N* hydrochloric acid was added. The test tube was then placed in a boiling water bath for 30 min. The solution was then cooled to room temperature, and 10 ml. phenol–hypobromite mixture (4) and sufficient distilled water to yield a total volume of 11 ml. were added. Forty minutes later, the absorbance of the solution at 625 nm. was determined spectrophotometrically. Reagent blank determinations were made by using water instead of urine in the procedure. A urine sample containing 2 mg. acetaminophen/100 ml. will yield a reagent phase with an absorbance of about 0.21/cm. pathlength by this method.

[1] Ether for Anesthesia, Merck, purified by successive washings with 1 *N* NaOH, 1 *N* HCl, and three washings with distilled water.

Table I—Verification of Assay Method for Acetaminophen and Its Metabolites and Salicylamide and Its Metabolites in Urine Samples Containing Both Groups of Substances

Metabolite[a]	Sample[b]	Concentrations, as mg. % APAP or SAM Equivalent: A — Urine after Acetaminophen Administration	B — Urine after Salicylamide Administration	Equal Parts, A and B: Assay	Equal Parts, A and B: Theoretical
APAP	I	1.6	0.0	0.8	0.8
	II	1.8	0.0	0.9	0.9
APAPG	I	10.4	0.0	5.0	5.2
	II	40.0	0.0	21.1	20.0
APAPS	I	9.2	0.0	5.0	4.6
	II	22.8	0.0	10.0	11.4
SAMG	I	0.0	8.6	4.3	4.3
	II	0.0	49.2	24.6	24.6
SAMS	I	0.0	10.2	5.1	5.1
	II	1.0	12.8	6.9	6.9

[a] Abbreviations: APAP, acetaminophen; APAPG, acetaminophen glucuronide; APAPS, acetaminophen sulfate; SAMG, salicylamide glucuronide; and SAMS, salicylamide sulfate. [b] Roman numerals refer to a specific urine sample obtained after either acetaminophen or salicylamide administration.

Table II—Recovery of Acetaminophen and Its Metabolites in Man[a]

Subject	Sex	Age, yr.	Weight, kg.	Dose, g.	Urinary Excretion Products,[b] % of Dose: APAP	APAPG	APAPS
Y	M	36	62	1	3.2	57.9	31.1
Y	M	36	62	2	3.4	65.3	29.3
J	M	26	85	1	3.1	62.5	20.5
K	M	24	66	1	3.7	63.7	25.8
K	M	24	66	2	3.5	63.4	24.1

[a] Acetaminophen in solution administered orally. [b] Abbreviations: APAP, acetaminophen; APAPG, acetaminophen glucuronide; and APAPS, acetaminophen sulfate.

Acetaminophen glucuronide was assayed as free acetaminophen after incubating 2 ml. urine with 1 ml. (5000 units) beef liver β-glucuronidase[2] and 3 ml. distilled water at 37° for 4 hr. Free acetaminophen was then extracted and assayed as described in the preceding paragraphs. Control experiments with pH 5.0 acetate buffer (0.2 *M*) instead of beef liver β-glucuronidase showed that nonenzymatic hydrolysis of acetaminophen conjugates was negligible (<1%) under the experimental conditions. Acetaminophen glucuronide concentrations were determined by subtracting the acetaminophen concentration obtained in the assay for free acetaminophen from the acetaminophen concentration obtained in the present assay.

Acetaminophen sulfate was determined as free acetaminophen after hydrolysis with sulfatase. Glusulase[3] containing 50,000 units of sulfatase and 100,000 units β-glucuronidase/ml., was diluted 10-fold with pH 5.0 acetate buffer (0.2 *M*). One milliliter of this solution and 3 ml. distilled water were added to 2 ml. urine, and this mixture was incubated at 37° for 4 hr. Acetaminophen was then extracted and assayed as described previously. Acetaminophen sulfate was calculated as the difference in acetaminophen concentration obtained in this assay and in that for acetaminophen glucuronide. Similar results were obtained with another enzyme preparation,[4] but this required a longer (>10 hr.) period of incubation.

Determination of Acetaminophen and Its Metabolites in the Presence of Salicylamide Metabolites—Gentisamide, a metabolite of salicylamide, interfered in the acetaminophen assay and had to be removed. Urine samples containing acetaminophen and salicylamide metabolites were adjusted to pH 9.0 with borate buffer, saturated with sodium chloride, and extracted with ether. Acetaminophen, but not gentisamide, is thereby extracted. Specifically, 2 g. sodium chloride, 5 ml. pH 9.0 borate buffer (0.2 *M*) saturated with sodium chloride, and 15 ml. washed ether were added to 5 ml. urine. The mixture was shaken for 15 min. and centrifuged. Ten milliliters of the organic phase was extracted with 1 ml. 0.1 *N* sodium hydroxide, and the subsequent procedures were as described for acetaminophen alone.

Acetaminophen glucuronide was assayed as acetaminophen after incubating 1 ml. urine with 2 ml. (10,000 units) β-glucuronidase and 2 ml. distilled water at 37° for 16 hr. To 2 ml. of this solution were added 1 g. sodium chloride, 8 ml. borate buffer saturated with sodium chloride, and 15 ml. washed ether. Acetaminophen was then extracted and assayed as described previously.

Acetaminophen sulfate was assayed as acetaminophen after incubating 1 ml. urine with 2 ml. of 10-fold diluted glusulase and 2 ml. distilled water at 37° for 16 hr. To 2 ml. of this solution were added 8 ml. borate buffer saturated with sodium chloride, 1 g. sodium chloride, and 20 ml. washed ether. Acetaminophen was then extracted and assayed as described previously.

Determination of Salicylamide and Its Metabolites—These determinations were carried out as described by Levy and Matsuzawa (1).

Individual urine samples were diluted with distilled water where necessary. All assays were carried out at least in duplicate. The sum of the assay results obtained from individual samples in a given experiment was compared to the results obtained by assaying a composite sample prepared by combining aliquots of the individual urine samples. These agreed within 5% or less. All samples were stored in a freezer until analyzed. Urine samples obtained from nonmedicated subjects produced no measurable blank values in the acetaminophen assay (<1 mg. per day).

RESULTS

The assay methods for acetaminophen, salicylamide, and their respective metabolites were checked for possible interference by the other substances. Thus, urine samples obtained after either acetaminophen or salicylamide administration were assayed, combined in equal volumes, and reassayed. This revealed an interference by gentisamide, a minor metabolite of salicylamide, in the acetaminophen assay. The assay procedure was therefore modified by adjusting the pH for extraction to 9.0, thereby separating the interfering substance from acetaminophen. Table I shows a representative example of analytical results which verify the assay methods employed in this study. The recovery of acetaminophen and its metabolites after oral administration of 1- and 2-g. doses of acetaminophen is summarized in Table II. The average recovery of drug in all forms was 92%, mostly as acetaminophen glucuronide and acetaminophen sulfate. Only about 3.4% of the dose was recovered as free acetaminophen.

[2] Ketodase, Warner-Chilcott, Morris Plains, N. J.
[3] Endo Laboratories, Garden City, N. Y.
[4] Mylase P, Nutritional Biochemicals Corp., Cleveland, Ohio.

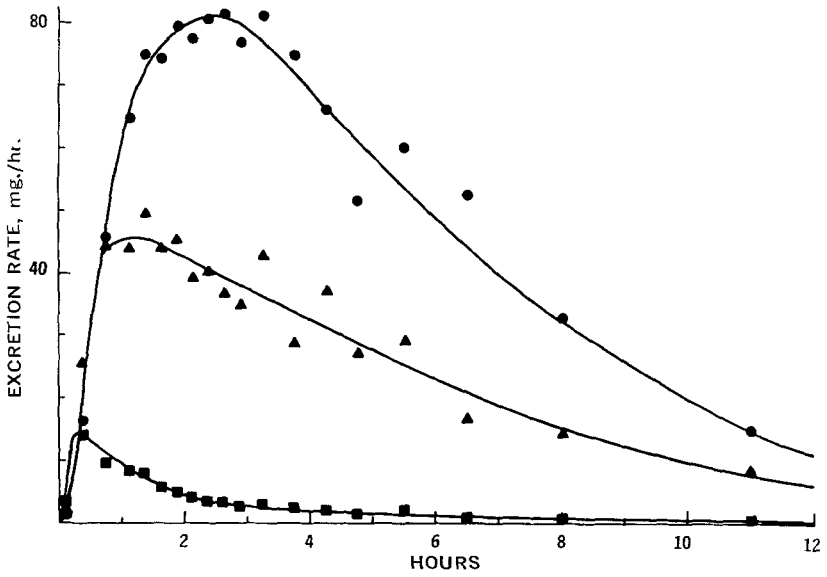

Figure 1—*Excretion rate of acetaminophen (■), acetaminophen glucuronide (●), and acetaminophen sulfate (▲) following oral administration of 1 g. acetaminophen to Subject Y. The excretion rate in this figure and in the subsequent figures is expressed in mg./hr. acetaminophen equivalent.*

Figure 1 shows the time course of urinary excretion of acetaminophen and its metabolites by Subject Y after oral administration of 1 g. of the drug. A semilogarithmic plot of acetaminophen excretion rate *versus* time could be resolved into two postabsorption exponential phases, suggesting an initial distributive phase (Fig. 2). Administration of 2 g. acetaminophen to the same subject resulted in the excretion of acetaminophen sulfate at an essentially constant rate for about 5 hr., this rate being only slightly higher than the peak excretion rate of acetaminophen sulfate after the 1-g. dose (Fig. 3). On the other hand, the maximum excretion rate of acetaminophen glucuronide after 2 g. acetaminophen (200 mg. acetaminophen equivalent per hour) was more than twice as high as the maximum rate after the 1-g. dose (80 mg./hr.). Subject J excreted acetaminophen sulfate at a much lower rate than Subject Y and showed an excretion rate plateau of over 4 hr. even with a 1-g. dose of acetaminophen (Fig. 4). No such plateau in the excretion of acetaminophen sulfate was apparent after administration of 2 g. acetaminophen to Subject K (Fig. 5).

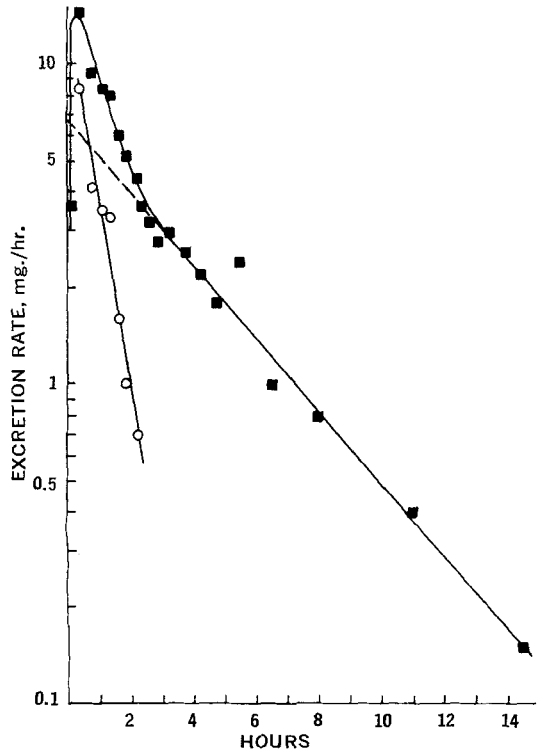

Figure 2—*Semilogarithmic plot of excretion rate of acetaminophen (■) following oral administration of 1 g. acetaminophen to Subject Y. The open circles represent the differences between the experimental values and the hypothetical values obtained by back-extrapolation of the terminal linear segment.*

One gram salicylamide, given 1.5 hr. after 1 g. acetaminophen, decreased the urinary excretion of acetaminophen glucuronide and acetaminophen sulfate for some time (Fig. 6). A more pronounced effect was obtained when the dose of salicylamide was increased to 2 g. (Fig. 7). The excretion rate of acetaminophen sulfate during the 1.5 hr. before salicylamide administration in these experiments was in good agreement with similar data obtained during the control experiments. This is particularly significant in the case of Subject J, whose excretion rate of acetaminophen sulfate was consistently low (Fig. 8). Concomitant administration of L-cysteine increased the excretion rate of acetaminophen sulfate and partly counteracted the effect of salicylamide (Fig. 9).

Acetaminophen decreased the formation of salicylamide sulfate from salicylamide, resulting in a somewhat increased formation of salicylamide glucuronide (Fig. 10, Table III). Concomitant administration of L-cysteine with salicylamide increased salicylamide sulfate formation and prevented the inhibition of salicylamide sulfate formation by acetaminophen (Table IV).

Table III—Effect of Acetaminophen on the Metabolic Fate of Salicylamide in Man[a]

	Recovery of Salicylamide Metabolites,[b] % of Dose			
	SAMG		SAMS	
Subject	Control	With APAP	Control	With APAP
Y	50.9	63.6	33.4	27.2
J	45.3	56.3	36.5	28.0
K	48.0	53.8	41.3	26.7

[a] One gram salicylamide administered orally alone or 1.5 hr. following 1 g. acetaminophen. [b] Abbreviations: SAMG, salicylamide glucuronide; SAMS, salicylamide sulfate; and APAP, acetaminophen.

Table IV—Effect of L-Cysteine on the Biotransformation Interaction of Acetaminophen and Salicylamide[a]

	Percent of Dose Recovered in Urine			
Metabolite	Control	With APAP	With L-Cysteine	With APAP and L-Cysteine
SAMG	50.9	63.6	40.2	41.8
SAMS	33.4	27.2	47.5	47.9

[a] One gram SAM alone or 1.5 hr. after 1 g. APAP. Two grams L-cysteine every hour from −1.5 to +2.5 hr. Abbreviations as in Table I, Subject Y.

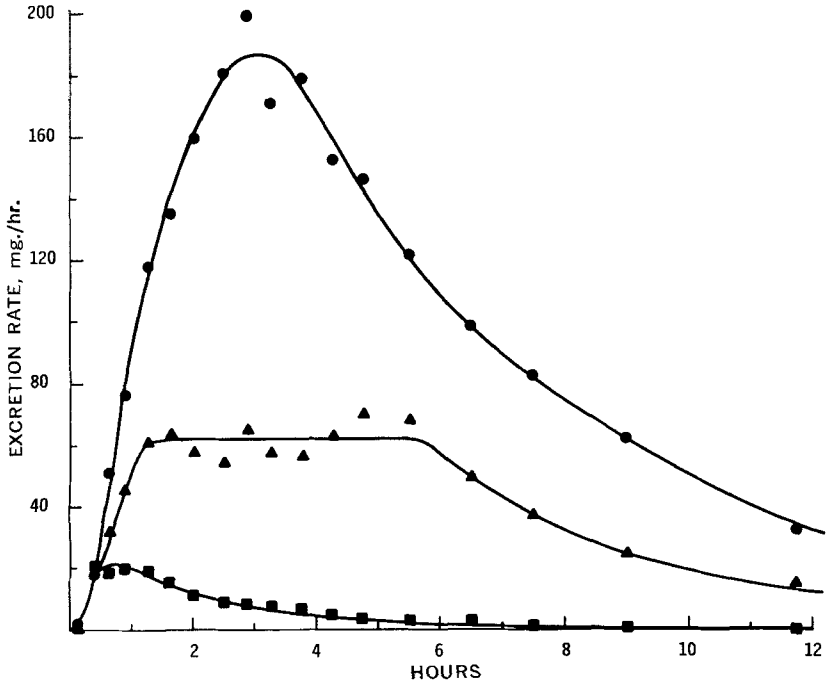

Figure 3—*Excretion rate of acetaminophen* (■), *acetaminophen glucuronide* (●), *and acetaminophen sulfate* (▲) *following oral administration of 2 g. acetaminophen to Subject Y.*

DISCUSSION

The total recovery of acetaminophen and its metabolites in this study (92% of the dose on the average) is similar to the average recoveries of 82 and 95% reported by Prescott *et al.* (5) and Nelson and Morioka (6), respectively. Cummings *et al.* (7) showed that acetaminophen is eliminated mainly as the glucuronide and sulfate and to a smaller extent as free drug. They identified the two metabolites by comparing their chromatographic characteristics and IR absorption spectra with those of pure acetaminophen glucuronide and acetaminophen sulfate. The composition of urinary excretion products of acetaminophen found in the present study (Table II) is very similar to that reported by Cummings *et al.* (7) (4% acetaminophen, 26% acetaminophen sulfate, and 49% acetaminophen glucuronide).

It appears that the formation of acetaminophen sulfate in man may be capacity limited in the 1–2-g. dose range. This is indicated by the acetaminophen sulfate excretion-rate plateaus following acetaminophen administration (Figs. 3 and 4) and by the less than proportional increase in acetaminophen sulfate excretion rates but more than proportional increase in acetaminophen glucuronide excretion rates when the dose of acetaminophen was increased from 1 to 2 g. (Figs. 1 and 3). While there were appreciable intersubject differences in acetaminophen sulfate formation, the results in any one subject were quite reproducible (Figs. 6–8). The fraction of acetaminophen excreted as acetaminophen sulfate decreased only slightly with increasing dose (Table II) in the dose range tested. This may be due to the fact that acetaminophen sulfate formation accounts for less than one-third of the acetaminophen elimination process and that deviation from apparent first-order acetaminophen sulfate formation may occur only when the amount of drug in the body is a relatively large fraction of the doses used in this study. There is also the possibility that acetaminophen glucuronide formation becomes capacity limited upon administration of the 2-g. dose of acetaminophen. In that case, the composition of the major urinary metabolites of acetaminophen would not change much with dose. Saturation of acetaminophen sulfate and acetaminophen glucuronide formation was observed in the rat by Büch *et al.* (8). They found that the same amount of acetaminophen sulfate was excreted within 4 hr. after administration of 300 and 600 mg./kg. acetamino-

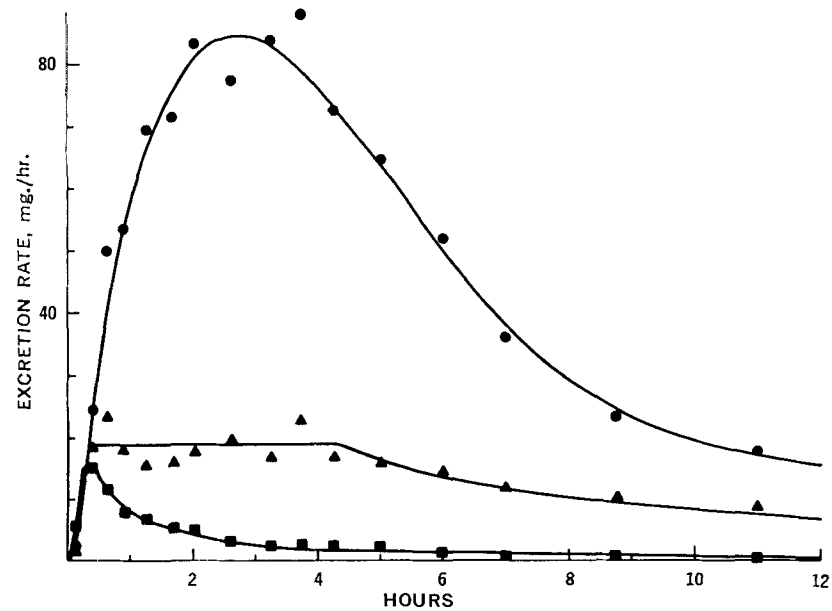

Figure 4—*Excretion rate of acetaminophen* (■), *acetaminophen glucuronide* (●), *and acetaminophen sulfate* (▲) *following oral administration of 1 g. acetaminophen to Subject J.*

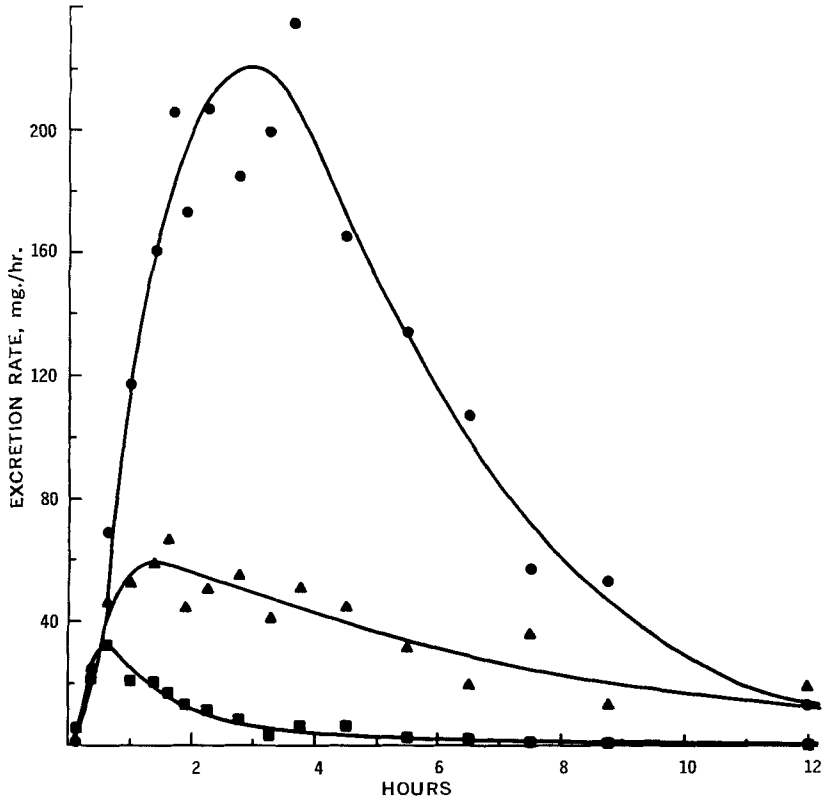

Figure 5—*Excretion rate of acetaminophen (■), acetaminophen glucuronide (●), and acetaminophen sulfate (▲) following oral administration of 2 g. acetaminophen to Subject K.*

phen and that acetaminophen glucuronide excretion increased by only 25%. The formation rate of acetaminophen sulfate could be increased by intravenous injection of sodium sulfate (9).

Concomitant administration of acetaminophen and salicylamide resulted in a mutual inhibition of sulfate formation (Figs. 6–8 and 10 and Table III). This effect was counteracted by L-cysteine (Fig. 9 and Table IV), a source of inorganic sulfate (10), which unlike inorganic sulfate is well absorbed upon oral administration. The formation of organic sulfate is a biphasic process: activation of inorganic sulfate to 3′-phosphoadenosine-5′-phosphosulfate followed by the transfer of sulfate to the acceptor molecule (11). The transfer reaction is mediated by a number of substrate-specific sulfokinases, while the activation step is common to each of these transfer reactions. Since the capacity-limiting step in the formation of salicylamide sulfate and acetaminophen sulfate appears to be the availability of sulfate, salicylamide and acetaminophen may be expected to inhibit competitively not only the formation of other phenolic sulfates but also the conjugation with sulfate of nonphenolic compounds such as various steroids which utilize different sulfokinases.

Based on the previously demonstrated competitive inhibition of glucuronide formation by salicylamide and salicylate (2), the decreased excretion of acetaminophen glucuronide after salicylamide administration (Figs. 6–8) is believed to be due to a similar competitive effect. However, the possibility that the renal clearance (rather than the formation) of acetaminophen glucuronide was inhibited by salicylamide or its metabolites cannot be excluded on the basis of the experimental data. The simultaneous inhibition of the parallel and competing process, sulfate formation, actually results in increased conversion of both acetaminophen and salicylamide to their respective glucuronides. Even the magnitude of a temporary inhibition of acetaminophen glucuronide formation rate would be "dampened" therefore by the more pronounced inhibition of the concurrent sulfate formation process.

This study showed not only that acetaminophen elimination may become capacity limited and that there occurs a mutual inhibition in

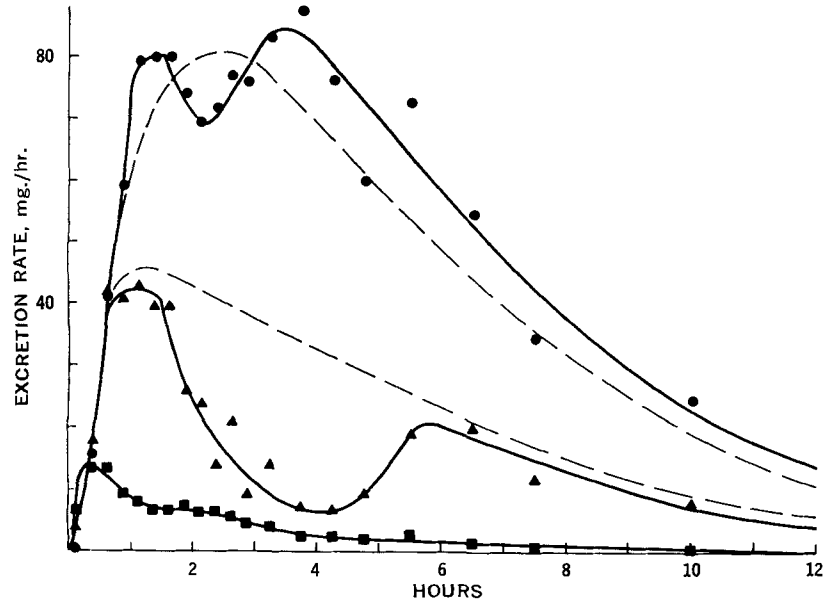

Figure 6—*Effect of 1 g. salicylamide, given at 1.5 hr., on the elimination of 1 g. acetaminophen by Subject Y. Key: ■, acetaminophen; ●, acetaminophen glucuronide; and ▲, acetaminophen sulfate. The broken line in this and the subsequent figures represents the excretion-rate curves of the corresponding substances following oral administration of 1 g. acetaminophen alone.*

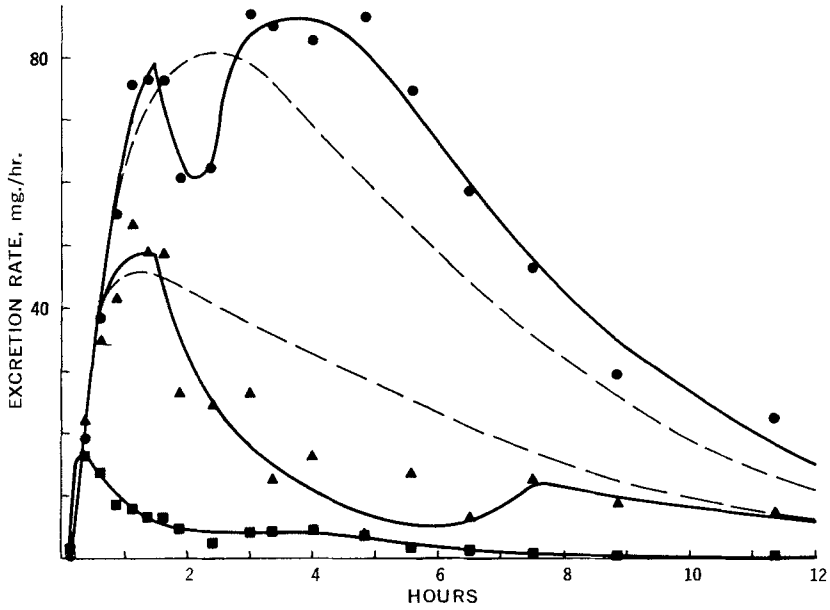

Figure 7—*Effect of 2 g. salicylamide, given at 1.5 hr., on the elimination of 1 g. acetaminophen by Subject Y. Key:* ■, *acetaminophen;* ●, *acetaminophen glucuronide; and* ▲, *acetaminophen sulfate.*

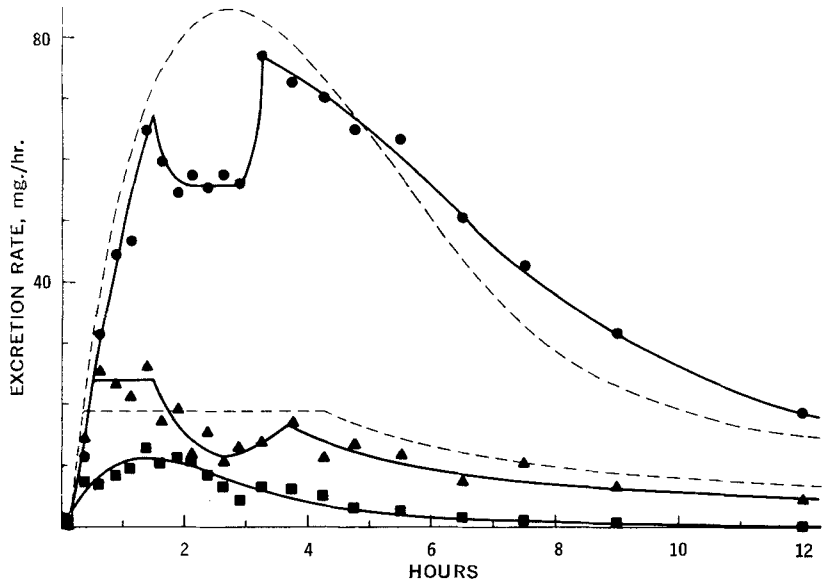

Figure 8—*Effect of 1 g. salicylamide, given at 1.5 hr., on the elimination of 1 g. acetaminophen by Subject J. Key:* ■, *acetaminophen;* ●, *acetaminophen glucuronide; and* ▲, *acetaminophen sulfate.*

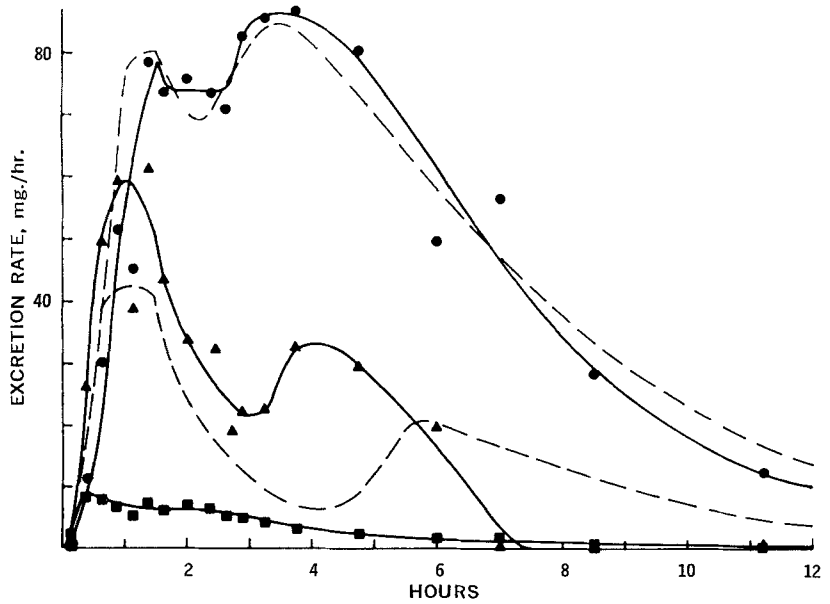

Figure 9—*Effect of a sulfate precursor* (L-*cysteine*) *on the influence of salicylamide on the elimination of acetaminophen by Subject Y. Two-gram doses of cysteine were administered at 0, 1, 2, 3, and 4 hr. The 1 g. acetaminophen was administered at zero time and 1 g. salicylamide was given 1.5 hr. later. Symbols are as in Fig. 1. The broken line is from Fig. 7 (acetaminophen and salicylamide).*

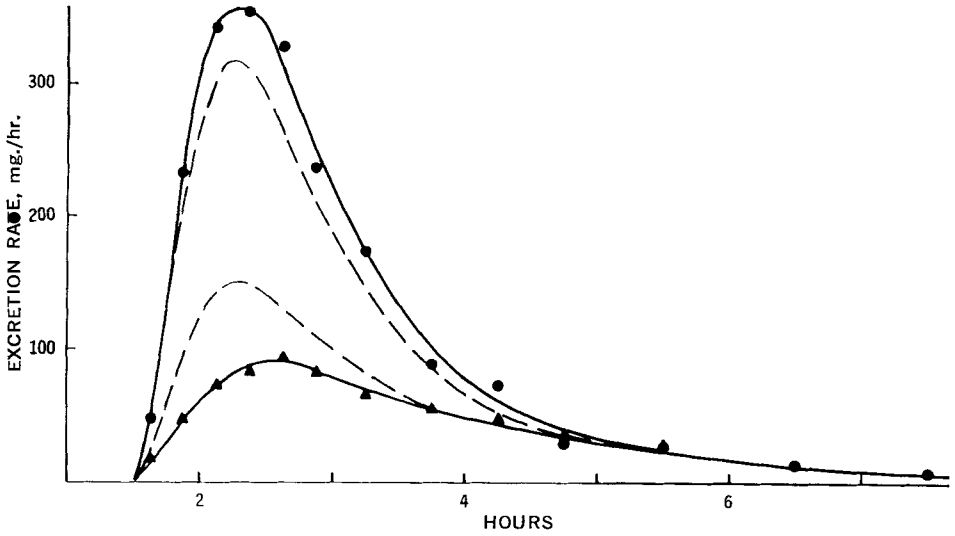

Figure 10—*Excretion rate of salicylamide glucuronide (●) and salicylamide sulfate (▲) following oral administration of 1 g. salicylamide at 1.5 hr. after 1 g. acetaminophen (Subject Y). The excretion rates are expressed as mg./hr. salicylamide equivalent. The broken lines represent the excretion-rate curves following oral administration of 1 g. salicylamide alone.*

the elimination of acetaminophen and salicylamide. It also revealed that, in the absence of noticeable saturation effects, acetaminophen elimination is a biexponential process. This feature of acetaminophen pharmacokinetics became apparent when the drug was administered in a rapidly absorbed form (solution) and when urine was collected every 15 min. (Fig. 2). Previous investigators, who administered the drug in solid form and collected urine less frequently, characterized acetaminophen elimination in terms of a one-compartment model (6, 7). If the results in Fig. 2 are reexpressed by combining several data points and thereby simulating results that would be obtained by collecting urine at greater intervals, the kinetics of acetaminophen elimination would agree perfectly with those obtained by other investigators (Fig. 11).

In this study, characterization of the previously reported biotransformation interaction between salicylamide and salicylate (2) has been extended to a description of the interaction of salicylamide and acetaminophen. The biotransformation of salicylate and acetaminophen in the presence of one another will be the subject of a subsequent report (12). These interactions between widely used non-narcotic analgesics have important pharmacokinetic and therapeutic implications (2, 13–15).

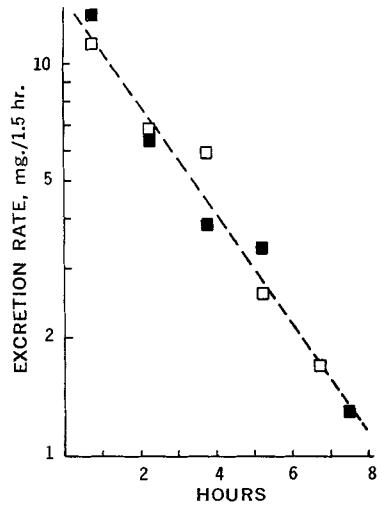

Figure 11—*Comparison of excretion rates of acetaminophen reported by Cummings* et al. *(Subject D, in Table I,* Reference 7*) with those of Subject Y in the present study. Key:* □, *Subject D from the study of Cummings* et al.; *and* ■, *data from Subject Y from Fig. 1 of the present study, but presented at greater intervals.*

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