fone concentration could be determined in the presence of 4-amino-4′-formamidodiphenylsulfone or 4,4′-diformamidodiphenylsulfone. However, the concentrations of 4,4′-diaminodiphenylsulfone, 4-amino-4′-formamidodiphenylsulfone, and 4,4′-diformamidodiphenylsulfone cannot be determined in a mixture of all three. As $k_1 > k_2$, then k' would be practically the same as the slower rate constant k_2.

The logarithmic version of the Arrhenius expression is:

$$\log k_2 = \log P - \frac{\Delta H_a}{2.303R} \cdot \frac{1}{T} \qquad \text{(Eq. 2)}$$

where ΔH_a is the energy of activation in cal. mole^{-1}, R is 1.987 cal. deg.$^{-1}$ mole^{-1}, T is the absolute temperature, and log P is the intercept of the plot of log k_2 against the reciprocal of the absolute temperature. The observed k_2 rate constants are given in Table I. The Arrhenius plots for the k_2 rate constants at various pH's are shown in Fig. 2. The ΔH_a values obtained from the slopes of Arrhenius plots for k_2 rate constants are listed in Table I. The k_2 rate constants at 25° at all pH's except pH 1.7 were calculated from the Arrhenius expression and are also included in Table I.

The log k_2–pH profiles for the hydrolysis of 4,4′-diformamidodiphenylsulfone at several temperatures are given in Fig. 3. At low pH (1.7), which is comparable to stomach pH, 4,4′-diformamidodiphenylsulfone is very rapidly hydrolyzed. At pH 4–6 the hydrolysis of 4,4′-diformamidodiphenylsulfone is much slower than at pH 1.7 (about 1/500). The hydrolysis is, however, enhanced at pH 7.85 and is about 5 times faster than at pH 4–6. For unknown reasons, at pH 9.65 the hydrolysis appeared to slightly decrease again. The results indicate that the hydrolysis of 4,4′-diformamidodiphenylsulfone is affected by pH and is first order with respect to 4,4′-diformamidodiphenylsulfone concentration. It may also be first order with respect to H^+-ion concentrations. 4,4′-Diformamidodiphenylsulfone is most stable at pH $\sim$ 6. Another arylamine substituted compound, *N*-acetyl-*p*-aminophenol, shows a similar log *k*–pH profile with a most stable pH $\sim$ 6 (10).

The present results agree with the *in vitro* and *in vivo* results reported by Gleason and Vogh (11) on the enzymic hydrolysis of 4,4′-diformamidodiphenylsulfone in mouse plasma. In their experiments, analysis of plasma showed a 4,4′-diformamidodiphenylsulfone undergoing a stepwise hydrolysis to 4,4′-diaminodiphenylsulfone through 4-amino-4′-formamidodiphenylsulfone as an intermediate.

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Drug Biotransformation Interactions in Man V: Acetaminophen and Salicylic Acid

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Abstract □ Orally administered salicylic acid (1 g. at −2 hr. and 0.5 g. at 4 hr.) had no significant effect on the formation of acetaminophen glucuronide and acetaminophen sulfate, or on the biologic half-life of acetaminophen in healthy adult volunteers who received a 1-g. oral dose of this drug. Acetaminophen did not inhibit the formation of salicylic glucuronides and salicylurate from salicylate. These results indicate that salicylamide is the major determinant in the previously reported biotransformation interactions between acetaminophen and salicylamide and between salicylate and salicylamide.

Keyphrases □ Salicylic acid—effect on acetaminophen, man □ Acetaminophen—influence of salicylic acid, man □ Biotransformations, man—acetaminophen–salicylic acid interaction

Previous studies in this series revealed mutual inhibitory effects in the biotransformation of acetaminophen and salicylamide to their respective glucuronides and sulfates (1), and of salicylic acid and salicylamide to their glucuronides (2). There was also some indication of an inhibitory effect of salicylate on the formation of salicylamide sulfate (2). To elucidate further the biotransformation interactions of these widely used analgesics and antipyretics, the effect of salicylate on the pharmacokinetics of acetaminophen was studied in normal adults. The results of this investigation will help to identify the major determinant in the previously described interactions, since each of the three interacting drugs has now been studied in the presence of the two others.

In addition to these mechanistic considerations, there has been some concern about a trend among pediatricians to prescribe full doses of aspirin and acetaminophen every 4 hr. so that the patient receives one or the other drug every 2 hr. (3). Acetaminophen is eliminated mainly by the formation of its glucuronide and sulfate (1). Salicylic acid is also subject to glucuronidation, and it inhibits the formation of at least some types of sulfates (2, 4, 5). Concern about the possible consequences of the

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

Metabolite[a]	Sample	Concentrations, as mg. % APAP or SA Equivalent			
		A: Urine after Salicylate Administration	B: Urine after Acetaminophen Administration	Equal Parts, A and B[b]: Assay	Equal Parts, A and B[b]: Theoretical
SA	1	1.6	0.0	0.8	0.8
	2	1.0	0.0	0.5	0.5
	3	1.3	0.0	0.7	0.7
SAG	1	16.6	0.0	8.3	8.3
	2	14.1	0.0	7.2	7.1
	3	18.6	0.0	9.3	9.3
SU	1	40.7	0.0	20.7	20.4
	2	42.5	0.0	20.5	21.3
	3	45.9	0.0	22.0	23.0
APAP	4	0.0	4.2	2.2	2.1
	5	0.0	2.0	1.1	1.0
	6	0.0	4.3	2.2	2.2
APAPG	4	0.0	17.5	8.8	8.8
	5	0.0	32.2	16.0	16.1
	6	0.0	68.7	35.1	34.4
APAPS	4	0.0	17.8	8.7	8.9
	5	0.0	22.1	11.3	11.1
	6	0.0	38.0	18.1	19.0

[a] Abbreviations: SA, salicylic acid; SAG, salicylic acid glucuronide; SU, salicyluric acid; APAP, acetaminophen; APAPG, acetaminophen glucuronide; and APAPS, acetaminophen sulfate. [b] Equal volumes of A and B were mixed in the following manner: 1 + 4, 2 + 5, 3 + 6.

concomitant use of full doses of acetaminophen and salicylate was therefore justified in principle and made a formal pharmacokinetic investigation desirable.

EXPERIMENTAL

Five healthy male volunteers, 27–33 years old, participated in this study. They received oral doses of 1 g. acetaminophen in one test, 1 g. acetaminophen and salicylic acid (1 g. 2 hr. before and 0.5 g. 4 hr. after acetaminophen) in the second test, and a total of 1.5 g. salicylic acid (1 g. initially and 0.5 g. 6 hr. later) in the third test. The drugs were given in aqueous solution (salicylic acid as sodium salicylate) in the morning on an empty stomach. Food was withheld for at least 2 hr. The tests were carried out in random order at approximately 1-week intervals. Urine was collected by three subjects every 0.5 hr. for 4 hr., every hour for 8–10 hr. thereafter, and then at longer intervals for a total of 24 hr. (acetaminophen alone) or 48 hr. (tests with salicylate). Two additional subjects collected urine at longer and irregular intervals. Their data are, therefore, not shown in the figures.

Acetaminophen and its metabolites in the urine were determined by the method described by Levy and Yamada (1) for the determination of acetaminophen and its metabolites in the presence of salicylamide, with the following modifications: (*a*) isoamyl acetate (analytical grade, Fisher) rather than ether was used for the extractions, since some manufacturers' ether, even if purified by repeated washing, interfered in the assay; (*b*) the borate buffer was saturated with sodium chloride and was prepared by adding hydrochloric acid to 0.16 *M* sodium tetraborate to apparent pH 8.0; and (*c*) all enzymatic hydrolyses were carried out for 16 hr. When separating the glusulase incubation mixture and isoamyl acetate by centrifuging, a gel phase formed at the aqueous–organic interphase. This gel was mixed gently into the aqueous phase with a stirring rod, and the system was then recentrifuged, resulting in a clean separation of the phases.

Total acetaminophen (free and conjugated drug) by acid hydrolysis was determined by adding 1 ml. concentrated hydrochloric acid and 2 ml. water to 3 ml. urine and heating this solution for 2.5 hr. at 100°. After cooling, water was added to yield a total volume of 10 ml. Five grams potassium phosphate (dibasic) was added to 5 ml. of the solution, and this was extracted with 10 ml. ethylene dichloride. Five milliliters of the organic phase was extracted with 5 ml. 2 *N* hydrochloric acid. To 1 ml. of the aqueous phase was added 10 ml. of phenol–hypobromite mixture (6), and the absorbance of this solution was determined at 625 nm. after 40 min. of color development.

Salicylic acid and its metabolites were determined as described by Levy and Procknal (2). Assay of acetaminophen and its metabo-

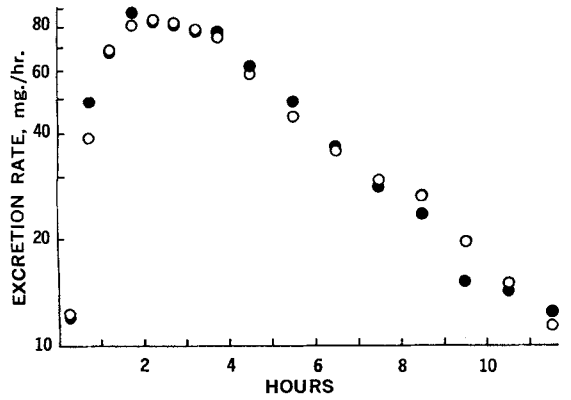

Figure 1—*Excretion rate of acetaminophen glucuronide (in terms of acetaminophen) as a function of time after oral administration of 1 g. acetaminophen alone (●) and with salicylate (○). Average of Subjects A, B, and C.*

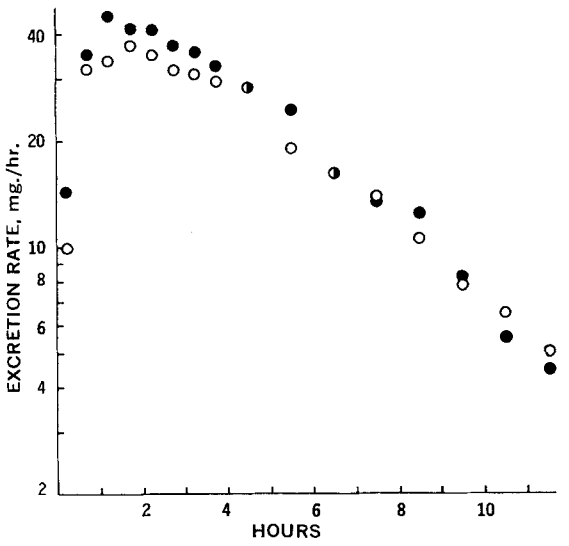

Figure 2—*Excretion rate of acetaminophen sulfate (in terms of acetaminophen) as a function of time after oral administration of 1 g. acetaminophen alone (●) and with salicylate (○). Average of Subjects A, B, and C.*

Table II—Effect of Salicylate on Biotransformation and Biologic Half-Life of Acetaminophen in Man[a]

Subject	Amount (and Percent)[b] Excreted, mg. APAP Equivalent										Half-Life, $t_{1/2}\beta$, hr.	
	Free APAP		APAPG		APAPS		Total (Glusulase)		Total (Acid)			
	Control	With SA	Control	With SA	Control	With SA	Control	With SA	Control	With SA	Control	With SA
A	18 (2.0)	29 (3.4)	685 (77.0)	681 (80.3)	187 (21.0)	138 (16.3)	890	848	878	849	2.2	2.2
B	33 (4.0)	50 (6.0)	497 (60.0)	518 (62.3)	299 (36.0)	263 (31.7)	829	831	862	878	2.8	2.8
C	15 (1.8)	23 (2.7)	484 (58.0)	464 (55.0)	335 (40.2)	357 (42.3)	834	844	803	846	2.5	2.5
D	26 (3.0)	24 (2.8)	590 (72.6)	640 (74.5)	199 (24.4)	195 (22.7)	813	859	815	827	1.6	2.2
E	32 (4.0)	25 (3.1)	593 (73.4)	583 (73.0)	183 (22.6)	194 (23.9)	808	802	799	827	1.8	1.8
Mean	25 (3.0)	30 (3.6)	570 (68.2)	577 (69.0)	241 (28.8)	229 (27.4)	835	837	831	845	2.2	2.3

[a] Abbreviations as in Table I. [b] Percent data are based on total recovery by glusulase hydrolysis and are listed in parentheses.

lites in urine samples obtained after acetaminophen administration, assay of salicylate and its metabolites in urine samples obtained after salicylate administration, and reassay of these substances in combined acetaminophen and salicylate urines showed that there was no interference by any of the substances in the analysis of the others (Table I).

RESULTS AND DISCUSSION

The time course of acetaminophen glucuronide excretion after acetaminophen administration was not affected by salicylate (Fig. 1). There was also no apparent effect on the time course of acetaminophen sulfate excretion (Fig. 2). Despite the very limited excretion of free acetaminophen (about 3% of the dose), semilogarithmic plots of free acetaminophen excretion rates *versus* time yielded a terminal linear (β-phase) segment which reflects the elimination of this drug from the body (Fig. 3). The average half-life of the β-phase was not affected by salicylate (Fig. 3 and Table II). Rapid absorption of acetaminophen by some subjects in some experiments also revealed an initial distributive phase as described previously (1).

Salicylate had no apparent effect on the terminal composition of urinary excretion products of acetaminophen and on the total recovery of this drug and its metabolites. Total acetaminophen was determined by enzymic (glusulase) as well as acid hydrolysis; the average results obtained by these methods differed by less than 1%. The three subjects represented in Fig. 3 excreted more free acetaminophen with salicylate than in the control experiments, but this was not the case with the other two subjects (Table II).

The dosage regimen of salicylate was designed to maintain relatively constant body levels of this drug over 12 hr. Since about half of a rapidly absorbed 1-g. dose of salicylate is eliminated by normal adults in 6 hr. (7), 1 g. was given initially and 0.5 g. was given 6 hr. later. Acetaminophen did not inhibit the formation and excretion of salicylurate and salicylic glucuronides (Fig. 4).

This investigation revealed no apparent interaction in the biotransformation of acetaminophen and salicylate. This does not rule out the possibility of interactions at higher doses or at elevated body levels attained after repeated dosings of these drugs. However, the ability of acetaminophen and salicylate to inhibit glucuronide and sulfate formation is clearly much less than that of equivalent doses of salicylamide, as is evident upon a review of the previous investigations in this series (1, 2). The pronounced competitive

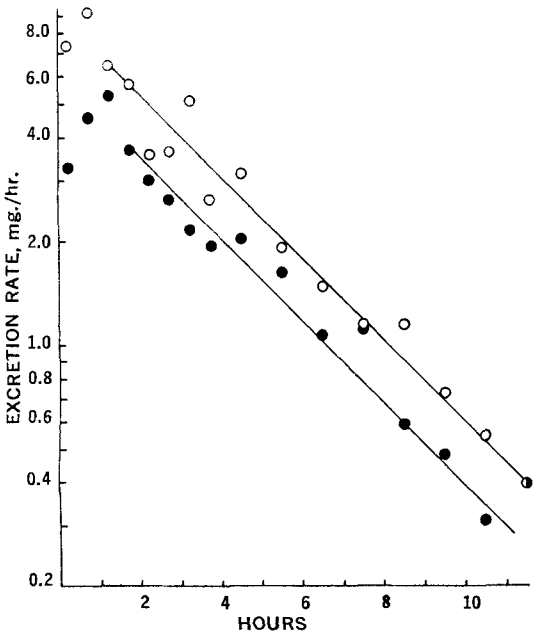

Figure 3—*Excretion rate of free acetaminophen as a function of time after oral administration of 1 g. acetaminophen alone* (●) *and with salicylate* (○). *Average of Subjects A, B, and C.*

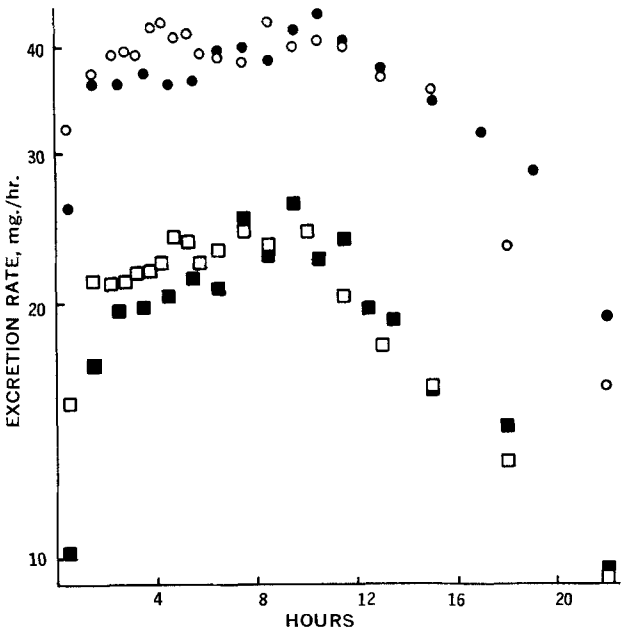

Figure 4—*Excretion rate of salicyluric acid (circles) and salicylic glucuronides (squares) after oral administration of 1 g. salicylic acid at zero time and 0.5 g. at 4 hr. All data are expressed in terms of salicylic acid. Closed symbols, control experiment; open symbols, with 1 g. acetaminophen given at 2 hr. Average of Subjects A, B, and C.*

inhibitory effect of salicylamide on glucuronide and sulfate formation is most likely due to its very rapid metabolism. The nonlinear nature of salicylamide elimination prevents comparisons of formation-rate constants, but an admittedly approximate estimation based on available data (1, 8, 9) indicates that the formation of salicylamide glucuronide and sulfate proceeds about 10 times more rapidly than the formation of acetaminophen glucuronide and sulfate at body levels of 1 g. of these drugs. It is now evident that of the several combinations of acetaminophen, salicylate, and salicylamide studied in this laboratory, salicylamide is the major determinant in the biotransformation interactions encountered (1, 2).

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MAO Activity in Normal, Cirrhotic, and Noncirrhotic Abnormal Human Liver

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Abstract □ Normal, cirrhotic, and noncirrhotic abnormal human livers from autopsy and biopsy specimens were analyzed for MAO activity. The enzyme activity was expressed on the basis of liver weight, liver nitrogen, and mitochondrial nitrogen. When MAO activity was expressed on the basis of liver weight, the cirrhotic liver showed 80% less activity and noncirrhotic abnormal liver showed 35% less activity than normal liver. Liver nitrogen and mitochondrial nitrogen seem to be equally valid as reference substances for MAO activity.

Keyphrases □ MAO activity—human liver, normal, cirrhotic, noncirrhotic abnormal, comparison □ Enzyme activity—human liver, normal cirrhotic, noncirrhotic abnormal, comparison □ Liver, human, normal, cirrhotic, noncirrhotic abnormal—determination, comparison of MAO activity

It is difficult to classify diseases of the liver since pathological conditions affecting the liver may arise from cell degenerative process, circulatory disturbance, necrosis, and tumor formation. Liver diseases can be characterized, however, by careful histological study of the tissue. Cirrhosis of the liver is best characterized by distorted reconstruction of lobules throughout the entire liver or in a considerable part of it. One widely believed theory concerning cirrhosis states that the degeneration of the liver cells results from an accumulation of toxic amines in the organ to a level that exceeds the deamination ability of the metabolic processes (1). The accumulation of toxic amines may be due to the lack of an adequate MAO activity. The purpose of this investigation was to study the activity of this enzyme system in normal, cirrhotic, and noncirrhotic but abnormal human livers.

EXPERIMENTAL

Human liver specimens were obtained from autopsy as well as by biopsy from hospital patients[1]. Liver specimens were stored in the freezer as soon as they were obtained. The specimens preserved in cold showed reproducible results (2). The isolation technique of liver mitochondria was similar to that reported by Hogeboom *et al.* (3).

In this procedure, carried out in a cold atmosphere, an approximately 0.7-g. portion of fresh or frozen liver was chopped into small pieces with a dissecting scissor onto a watch glass and transferred to a tared glass homogenizing tube; the exact weight of the sample was recorded. Homogenization of this chopped tissue was carried out in 5 ml. of 0.88 *M* sucrose solution for 5 min. The homogenate was quantitatively transferred to a 50-ml. polyethylene tube. The homogenizing tube and pestle were thoroughly rinsed with three or four portions of 0.88 *M* sucrose solution, and the rinses were added to the polyethylene tube containing liver homogenate. The final total volume of homogenate, representing approximately 20 ml., was then centrifuged (cold room 0–5°) at $600\times g$ for 10 min., and the supernatant fraction containing mitochondria and microsomes was transferred by means of a pipet into a 50-ml. polyethylene tube. The supernatant containing mitochondria was centrifuged at $24{,}000\times g$ for 20 min. to sediment the mitochondria, and the supernatant portion containing microsomes was discarded.

The solid mass of mitochondria was resuspended in 20 ml. of 0.88 *M* sucrose solution and centrifuged at $24{,}000\times g$ for an additional 20 min. This supernatant fraction was discarded. The packed mass of mitochondria was quantitatively transferred into a homogenizing tube, and the suspension of mitochondria in phosphate buffer pH 6.9 (0.5 *M*) was facilitated by careful homogenization. The mitochondrial solution was then transferred into a 10-ml. volumetric flask, the homogenizing tube was carefully rinsed with phosphate buffer, and the volume was made to the mark with

[1] University of Iowa General Hospital and Veterans Administration Hospital of Iowa City, Iowa.