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CONJUGATION KINETICS OF ACETAMINOPHEN BY THE PERFUSED RAT LIVER PREPARATION

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Abstract—Sulfate conjugation of acetaminophen was studied in the perfused rat liver preparation. Varying input concentrations of unlabeled acetaminophen (0.22 to 6.0 μg/ml or 1.5 to 40 μM) were added in a stepwise fashion once-through the liver preparation at a flow rate of 10 ml/min. For the concentration range tested, acetaminophen sulfate conjugate remained the only detectable metabolite in perfusate plasma. Other metabolites such as the glucuronide and the glutathione conjugates, however, were detected only in bile and, together with the sulfate conjugate and unconjugated acetaminophen, constituted 5 per cent of the total administered dose. Hepatic elimination of acetaminophen in the formation of sulfate conjugate was apparently maximal at input concentrations ≤1.0 μg/ml (6.7 μM) and could be viewed as mediated via a uni-enzyme system. Sulfate conjugation also decreased with preloading of the liver, especially with high concentrations of acetaminophen. A plot of the ratio of the steady-state output concentrations of drug to metabolite versus the reciprocal of drug extraction ratio, which might prove useful in the prediction of metabolite concentrations in the liver, was introduced.

The metabolism of acetaminophen, an analgesic, has been studied extensively. The phenolic group forms either sulfate or glucuronide conjugates. The proportions of sulfate and glucuronide conjugates formed from acetaminophen are highly age- [1, 2], species- [1, 3], and dose-dependent [3–5]. In the rat, sulfate conjugation is the main route of elimination for low doses of acetaminophen, both *in vivo* [6] and in the perfused rat liver preparation [7]. At high doses of the drug, however, the rate of sulfate conjugation is no longer a maximum for, and proportional to, the administered dose. Rather, it proceeds at a constant rate, and glucuronidation, which remains unsaturated, assumes a more dominant role.

Conjugation processes are intriguing as they represent detoxification mechanisms for most drugs yet, for some isolated examples such as the carcinogen 2-acetyl-aminofluorene [8], sulfation is a toxic pathway. Sulfate conjugation usually proceeds at a rate faster than glucuronidation, as the K_m for the substrate is lower. But at high concentrations of substrate, the rate of sulfation is suspected to be limited by the "active sulfate" pool or the amount of 3′-phosphoadenosine 5′-phosphosulfate (PAPS) that constitutes the rate-limiting step. Mulder and coworkers [9, 10] found that the addition of inorganic sulfate, a precursor of PAPS, to rats *in vivo* or to the perfused liver preparation improved the formation of harmol sulfate conjugates. Moldéus *et al.* [11] also showed that inorganic sulfate governs and regulates the degree of sulfation of both acetaminophen and harmol in the isolated rat hepatocyte. Schwarz [12] found that inorganic sulfate stimulated sulfate conjugation of 1-naphthol in isolated rat liver cells. Galinsky *et al.* [13] demonstrated that the pre-administration *in vivo* of inorganic sulfate to rats hastened the plasma clearance of acetaminophen in the form of sulfate conjugates. For this reason, it is highly possible that the administration of inorganic sulfate will stimulate the production of acetaminophen sulfate conjugates and, in turn, circumvent possible competitive toxic pathways.

To understand acetaminophen, which is used frequently and non-discriminantly, and whose toxicity is often cited [14], it is important to understand the kinetics of sulfate conjugation. Although dose dependence studies have been carried out which demonstrate a decrease in sulfation congruent with an increase in glucuronidation, the concentration/dose at which sulfate conjugation occurs maximally has not been elucidated. The present investigation is an examination of the kinetics of sulfate conjugation of acetaminophen in the perfused rat liver preparation. The concentration range over which sulfate conjugation occurs maximally for acetaminophen is also defined.

Some basic equations are used for the single passage of a drug through the liver preparation. The steady-state hepatic extraction ratio E, is defined as the difference in drug concentrations, measured at steady-state, that are entering (C_{In}) and leaving (C_{Out}) the liver, divided by the input drug concentration.

$$E = \frac{(C_{In} - C_{Out})}{C_{In}} \quad (1)$$

At low concentrations of acetaminophen, sulfate conjugation represents the predominant eliminatory pathway (> 95 per cent), and elimination can be assumed to be mediated by a unit-enzyme system, namely the sulfotransferases (the contribution of biliary excretion to the overall elimination of acetaminophen is insignificant). By mass balance, the rate of formation of the sulfate conjugate is equal

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to the rate of drug loss of acetaminophen across the liver at steady state:

$$Q[M] = Q(C_{In}-C_{Out}) \quad (2)$$

or

$$[M] = C_{In}-C_{Out} \quad (2A)$$

where $[M]$ is the concentration of the sulfate conjugate in liver. After substitution of equation 1 into equation 2A, the equation can be rearranged to give the ratio of the steady-state output concentration of acetaminophen to the concentration of the sulfate conjugate in liver as:

$$\frac{C_{Out}}{[M]} = \frac{1}{E} - 1. \quad (3)$$

Because the sulfate conjugate is not a substrate for hepatic metabolism, and because biliary excretion of the sulfate is only a small percentage of the amount formed, the concentration of sulfate conjugate in liver $[M]$ is the same as that in hepatic venous blood.

MATERIALS AND METHODS

General tritium ring-labeled acetaminophen (sp. act. 937.5 mCi/mmole) was purchased from the New England Nuclear Corp., Boston, MA. Acetaminophen sulfate conjugate and acetaminophen glucuronide conjugate were gifts obtained from McNeil Laboratories, Fort Washington, PA. Authentic acetaminophen and acetoacetanilide were purchased from the Eastman Kodak Co., Rochester, NY, and were recrystallized twice in methanol prior to use. Acetaminophen glutathione conjugate was obtained from Dr. Bernhard Lauterberg, Baylor College of Medicine, Houston, TX.

Male Sprague–Dawley rats (220–380 g), obtained from TIMCO, Houston, TX were fed *ad lib.* and were used as liver donors. The method of liver perfusion was identical to that described previously [7]. The commercially available TWO/TEN Perfuser (MX International, Aurora, CO) equipped with two reservoir units was used. The perfusate which contained 20% washed human red blood cells (Blood Bank, Houston, TX), 3% dextran (Pharmacia Fine Chemicals, Piscataway, NJ), 1% bovine serum albumin (Sigma Chemical Co. St. Louis, MO), and glucose (300 mg/100 ml) (Travenol, Deerfield, IL) in Krebs–Ringer bicarbonate solution buffered to pH 7.4 was delivered at a constant flow (10 ml/min) to the rat liver preparation. Unlabeled acetaminophen, together with a tracer dose of tritium-labeled acetaminophen, was added to reservoir perfusate and mixed thoroughly prior to delivery into the liver.

Only single-pass experiments were designed; the perfusate leaving the liver was not returned to the reservoir. Input concentrations were varied in a stepwise fashion (35- to 50-min intervals) through the same liver preparation. The mean of two determinations of perfusate taken before and during the perfusion period from the reservoir was used as the steady-state input drug concentration. The mean of five determinations of consecutive samples taken after 20 min of perfusion and at 4-min intervals from the hepatic venous blood after the attainment of steady-state was used as the steady-state output concentration. Bile was collected at 5-min intervals during the course of the experiment. The samples were frozen immediately at 0° prior to analyses.

Assay for radioactivity. Tritiated acetaminophen and its metabolites in plasma perfusate and in bile were assayed as described previously [7] by thin-layer chromatography (t.l.c.), with authentic acetaminophen, acetaminophen sulfate, and glucuronide conjugates as carriers. A volume of 100 μl of perfusate plasma and 10 μl of bile was applied directly onto Avicel F plates (250 μm Analtech, Newark, DE), which were developed in *n*-propanol–0.4 N NH_4OH (80:20, v/v). The regions were scraped and quantitated by liquid scintillation counting (Beckman, model LS 7500, Irvine, CA). Blood perfusate (1.0 ml) from reservoir and hepatic venous blood was also extracted into ethyl acetate as described previously [7]. Standards that contained known amounts of tritiated acetaminophen was extracted by the same procedure. The recovery of [^{3}H] acetaminophen was 78–90 per cent.

Assay for unlabeled acetaminophen. Perfusate (2–3 ml) from reservoir and outflow blood was placed in a 10 × 13 cm culture tube and assayed for acetaminophen by high performance liquid chromatography (h.p.l.c.). The extraction procedure included the addition of solid ammonium carbonate, 6 μg acetoacetanilide (0.2 ml of 30 μg/ml; Eastman Kodak Co.), the internal standard, and 5 ml ethyl acetate (Burdick & Jackson, Muskegon, MI). The contents were shaken in an aliquot mixer and centrifuged. The organic layer was transferred to a fresh 10 × 13 cm culture tube and evaporated to dryness under a stream of nitrogen. The residue was reconstituted by the addition of 200 μl methanol, and 40 μl of this solution was injected into the liquid chromatograph. Standards that contained 0.1 to 7.0 μg acetaminophen in blank perfusate were extracted by the same procedure.

A liquid chromatograph (Laboratory of Data Control, Riviera Beach, FL) equipped with a Constametric pump, a variable wavelength detector (Spectro-Monitor III), and a reverse phase column (Excalibar, Spherisorb 10 μm, 25 cm) was utilized. Methanolic solution (25%, glass distilled methanol, Burdick & Jackson) was delivered at a flow rate of 1.5 ml/min; the wavelength was set at 254 nm; the chart speed was run at 10 cm/hr. The retention times for acetaminophen and acetoacetanilide were 4.2 and 10 min respectively. The eluent collected in the interval between 3.5 and 6 min accounted for all the radioactivity associated with acetaminophen. The peak height of unlabeled acetaminophen (cm) was divided by the radioactivity (dpm) recovered for [^{3}H]acetaminophen for both the input and output samples.

RESULTS

Drug assay. The input and output concentrations for tritiated acetaminophen by t.l.c. and the blood extraction method were virtually identical. The t.l.c. procedure, however, separated acetaminophen from its metabolites both in perfusate plasma and in bile. Good correlations for acetaminophen in both methods existed because acetaminophen was unbound to perfusate components (K. S. Pang and J. R.

Gillette, unpublished results), and the blood to plasma ratio for acetaminophen was virtually unity.

The calibration curve for h.p.l.c. was linear over the range (0.1 to 7.0 μg) tested. The input concentrations of unlabeled acetaminophen (C_{In}) were determined with certainty from the calibration curve when the samples were processed on the same day. The internal standard tended to degrade on subsequent days, but because the specific activities of acetaminophen, as determined by h.p.l.c., in the input and output samples during the same perfusion period were identical, quantitation of output acetaminophen concentrations (C_{Out}) was determined by the blood extraction method and by t.l.c. and expressed in terms of radioactivity.

Long-term experiments. Four studies were performed, each with five periods of infusion to the same rat liver preparation. A representative experiment is depicted in Fig. 1. The input acetaminophen concentrations were varied from 0.22 to 6.0 μg/ml (1.5 to 40 μM), but the concentration of tritiated acetaminophen was kept constant throughout. The initial period was repeated at the end of the experiment to test the viability and stability of the preparation. The output concentrations of acetaminophen and the sulfate conjugate in perfusate plasma had apparently approached steady states, as indicated by the constancy of their values after 20 min of perfusion for each perfusion period (Fig. 1). The sulfate conjugate was the only metabolite detected; the glucuronide and glutathione conjugates were not present in detectable quantities. The steady-state hepatic extraction ratios for the different input acetaminophen concentrations varied from 0.722 to 0.651 (Study 1, Table 1). The elimination of acetaminophen was apparently first-order for input acetaminophen concentrations of less than 0.9 μg/ml; the steady-state extraction ratio remained maximal and constant, and the ratio of the output concentrations of acetaminophen to sulfate remained virtually identical. In this study, however, the rat liver preparation failed to rebound to the initial conditions, as evidenced by the decrease in the hepatic extraction ratio at the repeat of the initial conditions.

The biliary excretion of acetaminophen and its metabolites accounted for less than 5.4 per cent of the total dose. Acetaminophen (1.8 to 3.9 per cent) and the glutathione conjugate (2–3 per cent) accounted for small percentages of the excreted dose, while the sulfate (57–68 per cent) and the glucuronide (6–15 per cent) were major fractions. Plots of biliary excretion rates (Fig. 2) show that the rates with which acetaminophen and the glutathione and sulfate conjugates appeared in bile were constant after 20 min of perfusion for the first period and indicate that the binding/distribution processes for these drug species were completed by this time. But the time for excretion rate of the glucuronide conjugate to approach steady-state was longer.

The additional three experiments showed similar results for perfusate and bile (Tables 1 and 2, Studies 2 to 4). A trend in the elimination of acetaminophen can be discerned from the data. Sulfate conjugation remained the predominant pathway, and elimination appeared to be maximal and constant at input concentrations of acetaminophen ≤1.0 μg/ml (6.7 μM). Moreover, below this input concentration the ratio of steady-state output concentrations of acetaminophen to sulfate remained small and relatively constant.

Short-term experiments. Because the rat liver had failed to return to its original elimination rates, the short-term experiments (120 min, Studies 5 and 6) were done to test whether the viability of the liver decreased as a result of the long duration of perfusion (3 to 3.5 hr). In one experiment (Study 5; Fig. 3A), a low concentration (around 0.5 μg/ml) of acetaminophen was used for the first and third periods, while a high concentration (around 2.7 μg/ml) was used for the second period. In the second study (Study 6; Fig. 3B), the concentrations were reversed.

In the first study, the steady-state extraction ratios for acetaminophen at the low concentrations were 0.647 and 0.611, and at high concentration, 0.588.

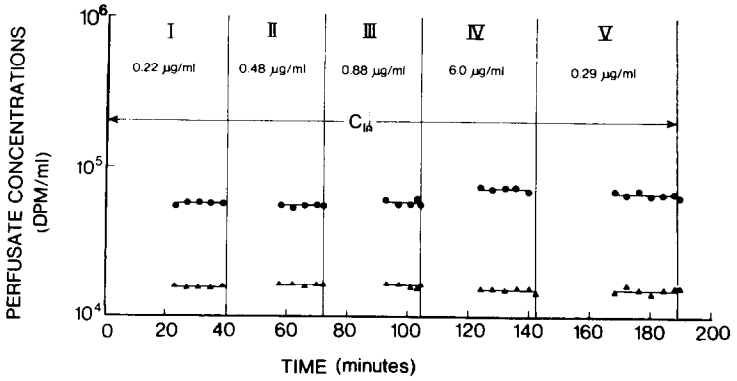

Fig. 1. Dose-dependent elimination of acetaminophen (unlabeled and tritium-labeled) by the perfused rat liver preparation. Varying input concentrations (0.22 to 6.0 μg/ml) of acetaminophen were delivered at 10 ml/min in five perfusion periods (I to V) to the same rat liver preparation. The constancy of the output concentrations of acetaminophen (●) and the sulfate conjugate (▲) indicated that the system had attained steady-state. C_{In} represented the steady-state input concentration of tritiated acetaminophen. The steady-state input concentration of unlabeled acetaminophen is also indicated for each perfusion period.

Table 1. Elimination of acetaminophen at different input concentrations of unlabeled and tritiated acetaminophen delivered at 10 ml/min once-through the rat liver preparations

Study	Steady-state input concentrations: Acetaminophen—C_{In} (μg/ml)*	Steady-state input concentrations: Acetaminophen—C_{In} (dpm/ml) $\times 10^2$†	Steady-state output concentrations: Acetaminophen—C_{Out} (d.p.m./ml) $\times 10^1$	Steady-state output concentrations: Sulfate conjugate	Ratio of output concentrations: Acetaminophen / Sulfate conjugate	Steady-state extraction ratio of acetaminophen (E)†‡
1	0.22	2041	580 ± 7§	1568 ± 38	0.366 ± 0.02	0.715
	0.46	2026	553 ± 3	1630 ± 36	0.339 ± 0.01	0.727
	0.88	2055	593 ± 27	1616 ± 48	0.367 ± 0.03	0.711
	6.0	2022	706 ± 24	1524 ± 19	0.466 ± 0.01	0.651
	0.29	2039	675 ± 23	1572 ± 65	0.430 ± 0.03	0.669
2	0.65	1768	475 ± 5	1264 ± 64	0.361 ± 0.04	0.731
	0.91	1769	472 ± 11	1311 ± 99	0.361 ± 0.03	0.733
	1.30	1799	537 ± 39	1270 ± 28	0.439 ± 0.01	0.701
	1.91	1820	637 ± 9	1222 ± 95	0.501 ± 0.03	0.650
	0.71	1800	538 ± 42	1228 ± 125	0.431 ± 0.03	0.701
3	1.07	2442	673 ± 52	1838 ± 225	0.357 ± 0.03	0.725
	1.20	2616	689 ± 66	2002 ± 128	0.338 ± 0.02	0.737
	1.82	2079	637 ± 44	1561 ± 34	0.409 ± 0.03	0.693
	3.0	2165	696 ± 40	1548 ± 86	0.447 ± 0.03	0.679
	0.98	2276	773 ± 40	1727 ± 96	0.446 ± 0.01	0.660
4	0.88	1666	748 ± 23	956 ± 43	0.783 ± 0.02	0.551
	3.00	1682	817 ± 92	914 ± 46	0.913 ± 0.09	0.514
	1.90	1538	703 ± 42	870 ± 75	0.811 ± 0.06	0.543
	1.54	1690	703 ± 65	873 ± 84	0.807 ± 0.04	0.563
	0.97	1552	601 ± 25	731 ± 36	0.828 ± 0.04	0.613
5	0.48	2508	886 ± 30	1603 ± 15	0.553 ± 0.02	0.647
	2.71	2502	1107 ± 14	1566 ± 38	0.707 ± 0.08	0.558
	0.54	2416	941 ± 28	1508 ± 62	0.627 ± 0.02	0.611
6	3.23	2538	944 ± 33	1615 ± 50	0.595 ± 0.03	0.628
	0.61	2400	915 ± 21	1563 ± 41	0.568 ± 0.01	0.626
	3.23	2393	978 ± 30	1506 ± 29	0.649 ± 0.02	0.591

* Assayed by h.p.l.c.
† Assayed by t.l.c.
‡ E is calculated as in equation 1.
§ Standard deviation.

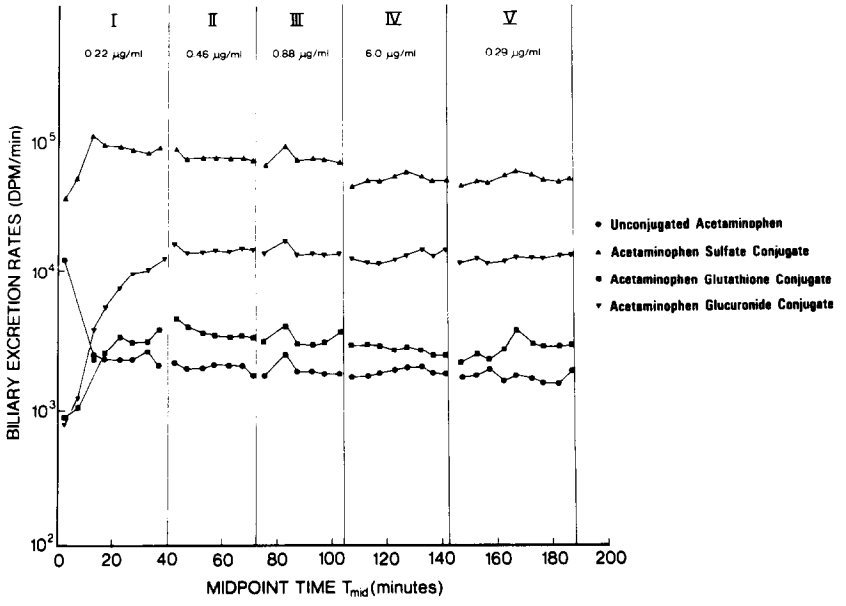

Fig. 2. Biliary excretion of acetaminophen and its metabolites by the perfused rat liver preparation under varying input acetaminophen concentrations. The biliary excretion rates of acetaminophen and its metabolites were plotted against the midpoint time of the collecting interval, T_{mid}.

Table 2. Biliary excretion of acetaminophen at different input concentrations of unlabeled and tritiated acetaminophen delivery at 10 ml/min once-through the rat liver preparations

Study	Input acetaminophen concn (μg/ml)	% Dose in bile	Percentage in bile*		
			Acetaminophen	Acetaminophen sulfate conjugate	Acetaminophen glucuronide conjugate	Acetaminophen glutathione conjugate
1	0.22	5.4	3.9	68.4	6.1	2.4
	0.46	4.9	1.8	62.0	13.2	3.2
	0.88	5.2	1.8	64.6	13.5	3.1
	6.0	4.5	1.9	57.1	14.0	2.6
	0.29	4.0	2.0	60.0	15.3	3.3
2	0.65	4.7	1.9	75.4	7.9	3.2
	0.91	4.5	2.3	53.4	16.7	4.9
	1.30	4.0	2.1	58.1	19.0	5.0
	1.91	3.7	2.2	53.1	18.3	3.9
	0.71	3.3	2.5	50.2	18.9	3.4
3	1.07	3.7	2.5	80.0	9.6	4.7
	1.20	3.8	2.4	63.2	18.1	4.8
	1.82	4.0	2.2	61.8	19.0	5.2
	3.0	3.2	2.5	59.8	20.3	4.9
	0.98	2.7	2.5	57.4	18.8	4.5
4	0.88	2.4	1.7	71.8	7.0	3.7
	3.00	2.8	2.5	52.0	20.4	6.1
	1.90	3.0	2.9	45.8	30.0	7.0
	1.54	3.0	2.9	43.7	32.5	6.8
	0.97	2.6	2.9	42.8	33.7	6.5
5	0.48	3.1	2.1	63.1	14.3	3.4
	2.71	4.6	4.2	58.8	21.8	4.1
	0.54	4.7	2.2	60.0	23.9	3.9
6	3.23	3.2	2.3	67.6	13.9	3.3
	0.61	3.3	2.3	61.3	23.0	5.0
	3.23	3.2	2.6	58.8	20.6	5.1

* Assayed by t.l.c.

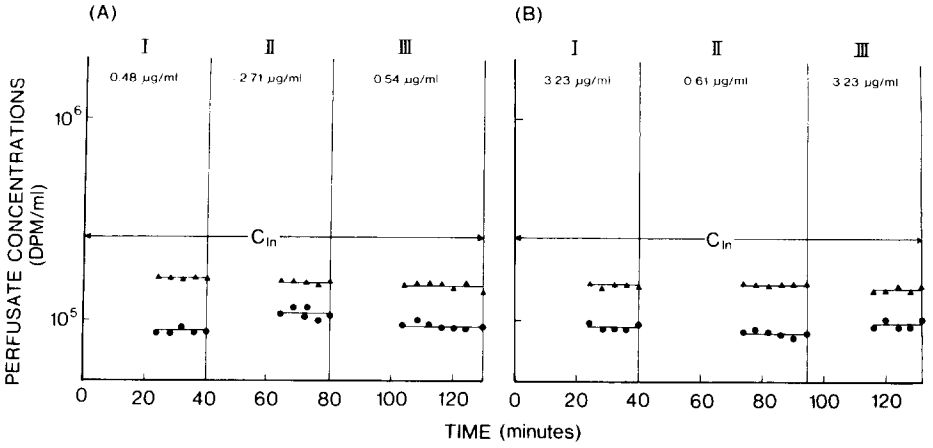

Fig. 3. Dose-dependent elimination of acetaminophen (unlabeled and tritium-labeled) by the perfused rat liver preparation. Varying input concentrations (log scale, around 0.5 and 3 μg/ml) of acetaminophen were delivered at 10 ml/min to two different rat liver preparations (A and B), with the order of the perfusing concentrations reversed. Symbols are identical to those in Fig. 1.

Moreover, the ratios of the output drug to the metabolite concentrations were smaller at the low concentrations (Study 5; Table 1). Biliary excretion data (Table 2) indicated that more acetaminophen and less sulfate conjugate were excreted at the high concentration. It appeared that the rat liver was partially capable of returning to the initial conditions at the third period. In the second experiment (Study 6), hepatic extraction ratio decreased in spite of the low concentration (Table 1, Fig. 3B), and no apparent trend was evident from the bile data (Table 2).

DISCUSSION

Biliary excretion of acetaminophen and its metabolites into bile constituted only a minor percentage of the total dose (5 per cent). The sulfate conjugate was the major fraction present (42 to 80 per cent), and attained a steady rate in excretion quite readily (Fig. 2). By constrast, the glucuronide conjugate (6.1 to 34 per cent) required a longer period of time to approach a steady-state. It is inconceivable that the sulfate and glucuronide differ drastically in binding/distribution characteristics within the liver, as both are quite polar drug species. In this respect, it appears that the formation of the glucuronide is slower than that of the sulfate, and steady state is reached later. For this reason, the first period of bile collection (the first 40 min of perfusion) contained less glucuronide conjugate and more sulfate conjugate than would occur in the steady state.

The biliary excretion rates and the proportions of unconjugated acetaminophen and the glucuronide were relatively constant with increasing concentrations (Table 2, Fig. 2). Decreases in biliary excretion rate and proportion of the sulfate conjugate, however, occurred with increases in acetaminophen concentrations (Table 2, Fig. 2). But the decrease in excretion of the sulfate conjugate was not compensated by an increase in excretion of either unconjugated acetaminophen or the glucuronide.

By contrast, perfusate plasma data were more revealing. A linear input concentration range of acetaminophen was identifiable in the system. At input concentrations ≤ 1.0 μg/ml (6.7 μM), sulfate conjugation represented virtually the sole route of metabolism for acetaminophen. The extraction ratios remained constant and maximal and the ratios of the output concentrations of acetaminophen to sulfate were constant and of the lowest value. This ratio of drug to metabolite output concentration can be viewed as an additional parameter that substantiates the extent of drug metabolism. A high E furnishes low drug and high metabolite (terminal metabolite) concentrations or a low drug-to-metabolite ratio. If E remains high, the ratio will have a low value.

The present findings with varying input concentrations of acetaminophen ≤ 40 μM failed to produce detectable amounts of glucuronide in perfusate plasma. This observation was similar to that obtained in our tracer concentration studies [6, 7, 15], when the elimination of acetaminophen proceeded maximally via sulfation, and the glucuronide appeared only in bile and constituted <1 per cent of the total dose. By contrast, Grafström *et al.* [16] detected as much as 20 per cent of the dose as acetaminophen glucuronide in perfusate and in bile at higher doses (1, 5 and 10 mM) in the recirculating rat liver perfusion system. The discrepancy may be due to differences in concentration/dose used and the nature of the experimental design. Direct sampling and the steady-state approach in the once-through perfusion system enabled pharmacokinetics parameters such as the extraction ratio and the steady-state concentrations of the drug and metabolite to be interpreted more easily.

The ratio of the steady-state output concentrations of drug to metabolite is quite useful. When the ratio was plotted against the reciprocal of the extraction ratio of the drug, a straight line (obtained by linear regression with no weighting of the data points) with a slope of 0.998 and a y intercept of -1.01 was obtained (Fig. 4). With the exclusion of the outlier, the slope and intercept were virtually unchanged (0.973 and -0.983 respectively). The plot correlated well with equation 3, which indicates that a slope of

unity and a y intercept of −1 should be obtained. It is suggestive, therefore, that the plot can be used to predict terminal metabolite concentrations when the extraction ratio and the steady-state output concentrations of a drug are known. As the equation is based strictly on mass balance, it will hold true for all dose levels without the assumption of any type of modeling as long as a uni-enzyme system exists within the liver for drug elimination. Further, in the case where the metabolite is sequentially metabolized at its time of formation before its initial entry into the systemic circulation [17], this plot will be useful for the estimation of the actual amount of metabolite formed from the drug. Although this concentration of the metabolite in the liver is easily ascertained by equation 2A, direct sampling of C_{In} and C_{Out} is not easily performed except in liver perfusion studies. Equation 3 proves to be more useful *in vivo* during chronic drug infusions where the systemic concentration of drug equals the hepatic venous concentration C_{Out} at steady-state [18], and the hepatic extraction ratio of the drug is estimated indirectly by the relationship: E = hepatic clearance/hepatic blood flow. In this respect, the steady-state concentration of the metabolite in liver can still be predicted adequately by equation 3.

The plot, constructed by data points that originated from six experiments, yielded a slope of 1 and a y intercept of −1 in spite of the fact that the viability and stability of the liver preparations changed during the time course of the experiment. Any decrease in the extraction ratio will result in an increase in the ratio of the output concentrations; less sulfate is formed and more acetaminophen emerges in the hepatic venous blood. It is true that the viability and stability of the preparation will influence the extraction ratio but, because of the dependence of the output concentrations of both acetaminophen and the sulfate conjugates on the extraction ratio, the correlation between the reciprocal of the extraction ratio and the ratio of output concentrations remains unchanged.

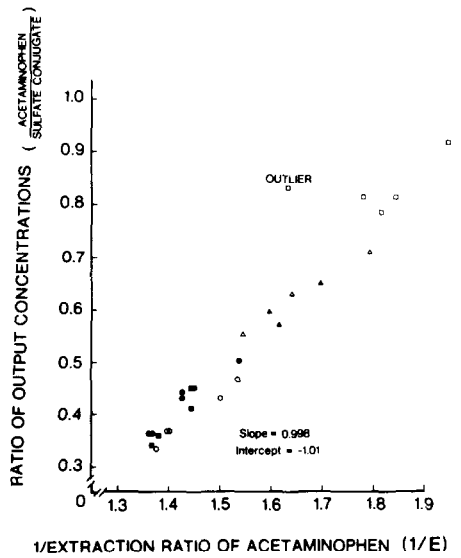

Fig. 4. Plot of the ratio of the steady-state output concentrations of acetaminophen to that of sulfate conjugates against the reciprocal of the steady-state hepatic extraction ratio ($1/E$) of acetaminophen.

The rat liver suffered apparent loss of stability over the time course of the experiment despite which the time of perfusion was shortened considerably (from 200 to 120 min). From previous experience [7, 15, 16], when liver perfusion was carried out for 120 min or 180 min, the viability of the liver was preserved and the stability loss, as in these studies, was not paralleled. Since the loss of stability occurred after preloading of the liver with acetaminophen, and sulfate conjugation remained virtually the only route of elimination, it is plausible that this metabolic pathway is partially inhibited by the depletion of PAPS, as suggested by other investigators [9–13]. Another possibility, the formation of a highly reactive metabolite which envelopes the sulfotransferases to prevent normal functionality (personal communication with Dr. J. R. Gillette), should not be excluded.

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